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EMBRYOGENY OF THE PODOCARPACEAE

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(WITH FIFTY-TWO FIGURES)

Introduction

The genus *Podocarpus* is divided, according to PILGER (**20**), into two subgenera —*Stachycarpus* and *Protopodocarpus*. The latter subgenus is further subdivided into several sections: Dacrycarpus, Microcarpus, Nageia, and Eupodocarpus. Several years ago the writer (**4**) contributed a series of stages in the embryogeny of several species of *Stachycarpus*. The embryogeny of *Dacrydium cupressinum* had been described earlier (**3**).

The present paper is concerned chiefly with the embryogeny of Dacrycarpus, Nageia, and Eupodocarpus. The embryogeny of additional representatives of the subgenus *Stachycarpus* and of *Phyllocladus* is described more fully than formerly, with special reference to details concerning the binucleate embryonic and apical cells. This paper records practically all the properly preserved embryological material which it has thus far been possible to obtain from conservatories in this country, from correspondents in the Southern Hemisphere, or from collections made by American botanists while abroad. I am indebted to Mr. H. W. Lawton of Wellington, New Zealand, for collections of *Podocarpus totarra* and several other species; to Dr. E. W. Sinnott for *P. dacrydioides;* and to Dr. M. S. Markle for specimens of *P. urbanii* obtained on Blue Mountain, Jamaica. The material of *P. macrophyllus maki* (*P. chinensis*) was collected by the writer from the conservatory of the New York Botanical Garden. While I have observed many species of the Podocarpaceae on estates and in parks and public gardens in California, very few of the species grown in this country, aside from *P. macrophyllus*, were found to produce seeds. Some in California produce pollen cones and ovules, but unfortunately many of the plantings of rare species are so scattered as isolated specimens that

the dioecious species lack the facilities for pollination. Some of the material of *Phyllocladus alpinus*, used previously by YOUNG (**27**), was found to include a number of stages in the embryogeny. With the exception of the latter, which had been sectioned, and some of the material of *P. totarra*, which was also cut serially, the material was dissected and stained following the methods described elsewhere (**5**).

The material of the Nageia section of *Podocarpus* and that of *P. gracilior* and *P. amarus*, belonging to the *Stachycarpus* subgenus, as well as that of a few South American podocarps, was all obtained from dried herbarium specimens. Fully enlarged ovules and seeds were cracked open so that the female gametophyte or endosperm could be removed and placed in water for a day or two. In many instances this tissue would swell by imbibition to its former size and shape, regaining its full turgor. The technique of dissection which has been described (**5**) for living or preserved material could then be used. In the instances in which the female gametophytes would not swell to their former size and shape, dissections were much more difficult but not impossible.

Fully enlarged ovules may be in stages before fertilization or have fully matured embryos. Only an occasional ovule will yield embryos in desirable early stages of development. As might be expected, the embryos obtained from dried material are usually somewhat shriveled and deformed. Usually considerable endosperm tissue adheres to the surface of the suspensor and embryonic tips, and this is difficult to remove. These tissues interfere somewhat with microscopic observation, and their complete removal is likely to damage parts of the embryonic structures. Nevertheless, after imbibition followed by staining with phloxine, the cell arrangement and number may be easily ascertained, and sometimes the nuclei may be observed; even chromosomes in late prophases of nuclear division may occasionally be recognized.

For the use of herbarium specimens, I am indebted to Dr. E. D. Merrill, Director of the Arnold Arboretum; Dr. Herbert L. Mason, Curator of the University of California Herbarium; Drs. Paul Standley and Julian Steyermark, Field Museum of Natural History; and Dr. J. M. Greenman, of the Missouri Botanical Garden.

The following list gives the species whose embryogenies are discussed or described here or previously, with some of the synonymy, following PILGER (**19, 20**) and in conformity with the 1930 and 1935 Amendments to the International Rules of Botanical Nomenclature.

Dacrydium cupressinum Sol. ex Forster . . . . . New Zealand (**3, 22**)
Podocarpus L'Herit. ex Pers.
Subgenus: *Stachycarpus* Engl.
P. spicatus R. Br. ex Bennett . . . . . New Zealand (**4, 22**)
P. ferrugineus D. Don . . . . . New Zealand (**4, 22**)
P. usambarensis Pilger . . . . . East Africa; cultivated in Jamaica (**4**)

P. gracilior Pilger Mountains of British East Africa; Uganda; Abyssinia
P. amarus Blume . . . . . Northeast Australia; Java; Sumatra; Philippine Islands; New Guinea

Subgenus: *Protopodocarpus* Engl.

Section I Dacrycarpus Endl.

P. dacrydioides Rich. . . . . New Zealand (**22**)
P. imbricatus Blume . . . . . British North Borneo and monsoon region

Section II Microcarpus Pilger . . . . . (no material obtained)

Section III Nageia Endl.

P. nankoensis Hayata . . . . . Formosa and Hainan
P. nagi (Thunb.) Zoll. & Moritz . . . . . Southern Japan; China (**26**)
P. blumei Endl. . . . . Sumatra; Philippine Islands

Section IV Eupodocarpus Endl.

P. urbanii Pilger . . . . . Jamaica
(*P. coriacea* Hook.) The Jamaica plant *P. coriacea* Hook. = *P. urbanii*, not *P. coriaceus* L. C. Rich.; probably not the plant collected by COKER (**8**)
P. macrophyllus (Thunb.) D. Don in Lambert . . . . Japan (**23, 26**); cultivated in California
P. macrophyllus maki Endl. . . . . Japan; cultivated in New Orleans and California; North Carolina to Florida
The plant collected by COKER (**8**) at Darlington, North Carolina, probably belongs here
(*P. chinensis* Wall. ex Endl.). This species, according to KÄMPFER, is often cultivated in conservatories of botanical gardens) . . . . . conservatory of New York Botanical Garden
P. glomeratus Don . . . . . Ecuador
P. coriaceus L. C. Rich. . . . . Venezuela; Puerto Rico
P. purdeanus Hook. . . . . Jamaica
P. matudai Lundell . . . . . Southern Mexico
P. totarra A. Cunn. (*P. totara* D. Don) . . . . . New Zealand (**22**)
P. hallii T. Kirk . . . . . New Zealand (**22**)
(*P. totarra* var. *hallii* [T. Kirk] Pilger)
P. nivalis Hook. . . . . New Zealand (**22**)

Phyllocladus alpinus Hook. f. . . . . New Zealand (**1, 13, 14, 27**)
Saxegothaea conspicua Lindl. . . . . Chile (**10, 17**); cultivated in British Isles

In the descriptions which follow, the Nageia group of *Podocarpus* is treated out of its order, since it is more convenient to describe this embryonic type last, together with several additional members of the *Stachycarpus* subgenus. This plan of treatment permits rendering the descriptions based on some of the dried ma-

terial by comparison with the figures obtained from properly fixed material. This order also avoids the necessity of including a larger number of illustrations.

Investigation

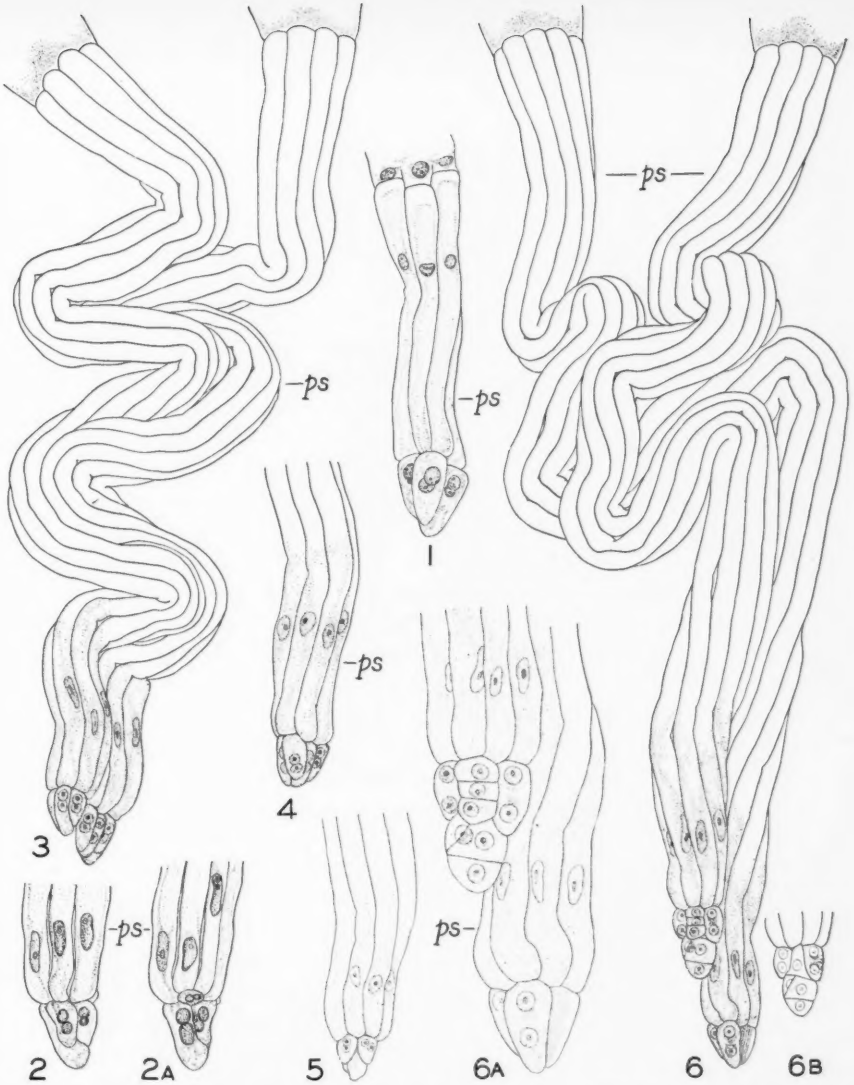
PROTOPODOCARPUS, SECTION DACRYCARPUS

PODOCARPUS DACRYDIOIDES.—The accompanying illustrations show a number of stages in the development of suspensor and early embryo of *P. dacrydioides*. These were dissected from preserved alcoholic material killed at Croydon, New Zealand, about January 30. While SINNOTT (**22**) covered many of the stages in the reproductive structures, including figures showing the sperm and egg nuclei side by side in fertilization, the proembryo of *P. dacrydioides* beyond the first division of the zygote was not included. The earliest stage obtainable from dissected material is shown in figure 1, in which the prosuspensor has begun to elongate but in which a tier of relict nuclei is still found above it. The embryo system shown in this figure has seven or more elongating cells in the prosuspensor, bearing four binucleate cells below at the tip. Figure 2 is from an embryo with a much longer prosuspensor, made up of nine cells, which bears five binucleate cells at the tip; figure 2, a surface view, shows only three of the embryo initials, while figure 2*a* of the same embryo group in the lower plane of focus reveals two other binucleate cells, one of which is located in an unusual position between the prosuspensor cells and the others. The binucleate cells are probably organized in the proembryo and may be regarded as actual or potential embryo initial cells.

In both of these embryo systems one of the embryo initials is much larger than the others. The largest may be located either at one side, as in figure 1, or more nearly in the center of the group. In early stages all binucleate cells are in contact with the prosuspensor at some point; that is, they usually form part of a single tier.

Figure 3 shows two neighboring embryo systems with prosuspensors twisted in the characteristic manner, the one at the left with three embryo initials and the one at the right with four. By the time this stage is reached the groups of embryonic initials are deeply imbedded in the female gametophyte, but they still remain as binucleate cells. In figure 4, the lower end of a prosuspensor composed of about ten elongated cells bearing five embryonic units is shown. In figure 5, the largest one of the group of five embryo initials has become aborted. Its disintegrating contents stain more deeply and traces of the nuclei are no longer observable.

Figure 1 shows the free nuclei still in the base of the egg above the prosuspensor, forming a tier of relict nuclei, while figures 3 and 6, showing the upper ends of several prosuspensors, indicate a complete absence of rosettes. Rosette cells could



FIGS. 1–6.—*Podocarpus dacrydioides:* Fig. 1, earliest observed stage showing tier of relict nuclei above prosuspensor (*ps*) bearing four binucleate cells; ×150. Fig. 2, surface view of 3 of embryonic group of binucleate cells. Fig. *2a*, optical section showing cells in deeper plane of focus; ×120. Fig. 3, 2 embryo systems, 3 binucleate cells borne on 9-celled prosuspensor at left, 4 or 5 units on 10-celled prosuspensor at right; ×120. Fig. 4, lower part of prosuspensor having 11–12 cells above, 9 below (where it bears 4 binucleate cells); ×120. Fig. 5, lower part of prosuspensor bearing 5 units with largest central unit dead; ×120. Fig. 6, embryo systems, 4 binucleate cells at right, 5 embryonic units at left; ×120. Fig. *6a*, tips of same; ×240. Fig. *6b*, largest 4-celled unit still in contact with prosuspensor.

be observed only rarely in any of the embryos successfully dissected. Figure 6 represents two adjacent embryo systems in a stage slightly older than those in the preceding figure. The embryo system at the right, which may have been more deeply imbedded, shows one of the four embryonic cells aborted. The other embryo initials in this group are still binucleate. The embryo system above at the left has just reached the stage in which some of the five embryo initials are replaced by groups of walled cells. Some of the cells which remain binucleate in both embryo systems of figure 6 show faint spindle fibers between the nuclei. The binucleate stage seems to represent, as shown by the persisting spindle fibers, a long-delayed telophase. Wall formation does not take place for many days after actual separation of the nuclei. The length of this interval is shown by the amount of elongation of the prosuspensor between a proembryo—or an embryo younger than figure 1—and one of the stage shown in figure 6.

Figure 6*a* is a more highly magnified view of the lower end of the two systems shown in figure 6. Figure 6*b* shows the arrangement of the cell walls in the upper or left embryo of figure 6 as this appears in a lower focus, indicating that a terminal 4-celled embryo has now been formed, and that this largest unit, as well as each of the other embryonic units, is still in contact with the prosuspensor. One of the other embryonic units has become 3-celled, and the two on the opposite side, masked by the three which are shown, are dead. This is the oldest stage observed in *P. dacrydioides*.

In the steps which follow figure 6 it is probable that the largest embryonic unit, which becomes the more protruding terminal embryo, pushes out a suspensor which thrusts the others upward with the prosuspensor. Several of the embryos may do this, more or less simultaneously, a condition which would produce for a time a double embryo or one of several lobes, until one of the tips ultimately outgrows the others. The general situation indicates that the embryogeny of *P. dacrydioides* has cleavage polyembryony, and there is considerable resemblance in the embryogeny, thus far, to that of *Dacrydium*, after which this species of *Podocarpus* was named. A slight difference comes from the fact that in *P. dacrydioides* all embryonic units are in contact with some part of the prosuspensor, while *Dacrydium* (3) usually has two tiers of these terminal embryo initials, only one of which touches the prosuspensor.

There may be five, four, or a smaller number of embryonic units remaining by the time cell proliferation of the individual embryos is initiated. In fact, several may become aborted during the observed stages of development. Figure 6*a* shows that they develop independently of one another, and the probability is that the embryo which ultimately survives is derived from only a single one of the binucleate embryonic units.

The writer has characterized the type of cleavage polyembryony in *Dacrydium*

as determinate (3). That of *P. dacrydioides* seems to be similar but somewhat less definite. The successful embryo, though larger at an early stage, may not always be situated in a terminal position.

The cells of a prosuspensor do not all extend to the embryonic units below. Several may drop out, so that an embryo system such as the one shown in figure 6 may have nine or ten elongated prosuspensor cells at the upper end and only seven or eight at the lower end, where their nuclei are usually located, with the ends of the short prosuspensor cells hidden or completely inclosed by the others. Rosette cells were rarely observed. In only one embryo system out of a dozen examples was a rosette cell shown, and this had not divided.

In the 4-celled embryo of figure 6, one of its cells remains in contact with the prosuspensor, indicating that all the several binucleate units were situated in the same plane or tier below the prosuspensor. After this 4-celled embryo has greatly increased the number of its cells, a massive secondary suspensor may be expected to elongate from its distal end, pushing the smaller embryonic units and prosuspensor upward.

PODOCARPUS IMBRICATUS.—The embryogeny of *P. imbricatus* is similar to that of *P. dacrydioides*. The only material available came from herbarium specimens. Three or four binucleate cells, arranged as in figures 1–5, were observed on the end of a prosuspensor of about nine or ten cells. In a later stage a larger terminal embryo was found on the end of a massive secondary suspensor, with a few smaller embryos placed laterally at various levels along the side of this suspensor system. This arrangement indicates determinate cleavage polyembryony in a stage later than that shown in figure 6; otherwise there was nothing to indicate important variations from the preceding account for *P. dacrydioides*.

PROTOPODOCARPUS, SECTION EUPODOCARPUS

The proembryo of a member of this group of podocarp species has been described by COKER (8) and more recently by TAHARA (26). From correspondence with DR. COKER, including an exchange of specimens, it appears fairly certain that the plant whose embryogeny he described belongs to a species very close to *P. macrophyllus maki*, under which PILGER (19, 20) places as a synonym the *P. chinensis* frequently grown in conservatories. It is certain that COKER'S species could not have been *P. urbanii* Pilger, under which *P. coriacea* Hook. appears as a synonym. The seeds of COKER'S species are much too large and agree more closely with *P. macrophyllus maki* (*P. chinensis*) than with *P. coriaceus* Rich., which I have examined from collections obtained at Maricao, Puerto Rico, and herbarium specimens collected in Venezuela.

PODOCARPUS URBANII.—The proembryo of this species has not been observed. From the earliest observed stages in the embryo system, it must be assumed that

the proembryo is organized with two or three terminal binucleate cells borne on the end of a prosuspensor made up of 12–14 cells.

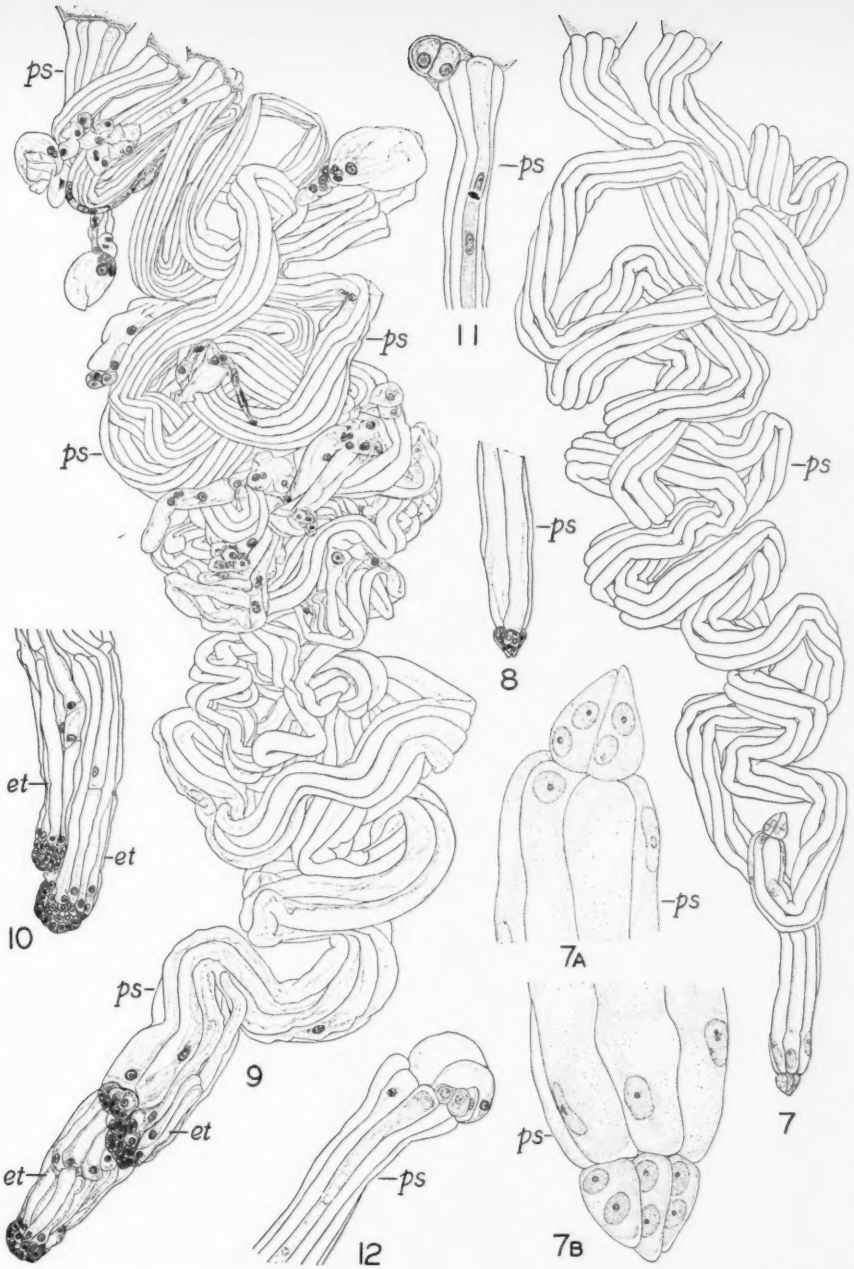
Figure 7 shows the earliest stage obtained from dissected material of *P. urbanii* collected in Jamaica. Two embryo systems are shown in which the prosuspensors have elongated greatly. The prosuspensor consists of about 12–14 cells at the top or base of the archegonium. These cells elongate together. As COKER (**8**) pointed out, some cells do not elongate fully, so that in figure 7 only seven or eight cells extend the entire length. The cells of the prosuspensor that drop out are frequently situated in the interior. In later stages some of these ends become free (fig. 9) and may form embryos on their ends.

There is a slight difference between *P. urbanii* and *P. macrophyllus maki* (*P. chinensis*). The former bears two or three binucleate embryonic cells (fig. 7*a*, *b*) while the latter bears only one or occasionally two. This is another feature wherein COKER'S species (**8**) seems to agree more closely with *P. macrophyllus maki* than with *P. urbanii*. Whether the binucleate cells are two or three, they were so organized in the proembryo. There are 3–5 binucleate cells in the very early embryo of *P. dacrydioides* (fig. 1), 9–10 in *P. usambarensis* (**4**), and—as TAHARA has shown (**26**)—7 or more in *P. nagi*, where they originate from the division of nuclei in walled cells of the proembryo.

Figure 8 shows one of these embryonic tips in a later stage in which the 4-celled groups belonging to each of three units are still distinctly recognizable. A similar figure more highly magnified, with each of the three embryonic groups containing about sixteen cells, is shown in figure 18. Figures 13 and 14, also more highly magnified, show 2- and 3-lobed multicellular embryos of similar and later stages. These are still attached to the prosuspensor (though in figure 14 the largest embryo is forming a massive secondary suspensor), while in figures 10, 15, and 17, all at lower magnifications, the secondary suspensor has elongated considerably.

In the 2- and 3-lobed terminal embryos, one of the cell groups has grown faster than the others. It is likely that only a single cell group or lobe will ultimately contribute the single embryo that survives in the mature seed. Some of the individual units seem to possess an apical initial cell which persists for a brief period, at most up to the stage of an embryo of 40–60 cells. The apical cell is not easily recognized in surface views (as in these drawings) but is usually more distinct in optical section.

Figure 9 is an older stage of *P. urbanii* in which three embryo systems are found. The three terminal embryos have all become separated in this case, owing to unequal growth of their respective secondary suspensors, while the terminal embryos belonging to the other two systems have become disorganized as the ends of their prosuspensor cells have become separated. The cells of the prosuspensor that do not elongate fully may become more or less dissociated. Where their ends



FIGS. 7–12.—*Podocarpus urbanii:* Fig. 7, 2 embryo systems with prosuspensors bearing 2 and 3 binucleate cells (×66); shown in fig. 7*a* and fig. 7*b*, ×290. Fig. 8, tip of similar system bearing 3 4-celled embryos. Fig. 9, 3 systems, longest with 3 embryos developing massive secondary suspensors (*et*), 2 other systems with terminal embryos unrecognizable and many small embryos formed on ends of prosuspensor cells; ×70. Fig. 10, tip of embryo system with 2 terminal embryos on massive secondary suspensors of embryonal tubes (*et*); ×70. Figs. 11, 12, upper ends of prosuspensors showing embryos in rosette region; ×140.

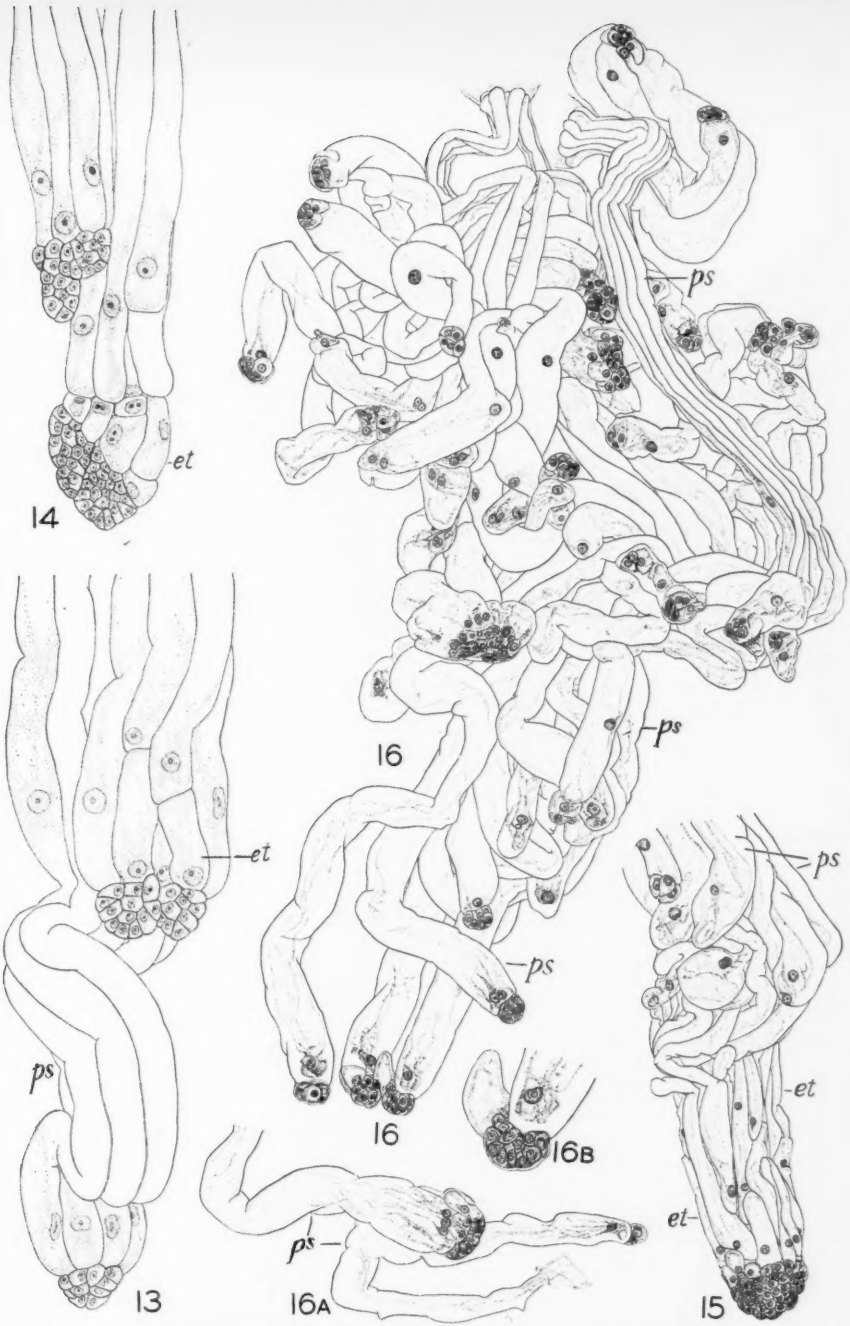
emerge from the tangle of elongated cells they may enlarge considerably and cut off at their tips one or more cells which develop into small secondary embryos. Many of them are shown in the upper half of figure 9. Figure 16 shows two systems of embryos which have disorganized into many smaller embryos, and the terminal ones shown below are not so large as in figure 9. The secondary embryos formed on the ends of prosuspensor cells therefore appear to have a primary suspensor. However, a true primary suspensor (single elongating cell given off by an embryo) is not found in this species. Thus the embryogeny of *P. urbanii* has cleavage polyembryony through the occurrence of secondary embryos formed on prosuspensor cells, as well as from the individual binucleate cells borne on the end of the prosuspensor. Some details of the manner in which the ends of prosuspensor cells form embryos will be shown in the embryogeny of *P. macrophyllus maki* which follows.

Figure 16*a* includes one of the largest embryos belonging to figure 16. This was broken off during dissection and may be derived from one of the terminal binucleate cells. Two others situated at the lower end of figure 16, both belonging to the system at the right side of the figure, may have come from a terminal cell. Figure 16*b* is an optical section at higher magnification, showing the internal arrangement of cells in relation to the apical cell. The terminal embryos belonging to the embryo system at the left of figure 16 are not recognizable with certainty, but are probably included among the many embryos in the upper half of this figure.

A total of twenty-six or more embryos is shown in figure 16. There are still more, but a number of them are situated beneath other structures. There are more than thirty derived from two fertilized eggs. The number of embryos of all kinds is therefore about fifteen per system, or nearly one for each prosuspensor cell plus two or three derived from the terminal binucleate cells shown in figure 7*a* and *b*.

The condition most frequently found is that shown in figure 9. The extreme cleavage polyembryony shown in figure 16 was found in about 10 per cent of the ovules of *P. urbanii*, and in a majority of the preparations there was some cleavage polyembryony, since some of the secondary embryos were found on the isolated ends of prosuspensor cells.

Figures 9, 10, 13, 15, and 17 show various stages for the terminal embryos in which massive secondary suspensors have appeared. A few embryos larger than these were obtained, but none were in the stage showing the organization of the meristems which give rise to the root tip, stem tip, and cotyledons. Figures 11 and 12 show the upper parts of prosuspensors with embryos formed in the position of rosette cells. Their origin is obscure. Sometimes the nuclei of prosuspensor cells, especially those that stay short, may remain in this upper region. Figures 11 and 12 both show this condition, also some of the upper ends of the prosuspensors in figure 9. There is a possibility that embryos may arise from the upper ends of



FIGS. 13–16.—*Podocarpus urbanii:* Figs. 13, 14, many-celled embryos on tips of prosuspensors, derived from 2 and 3 binucleate units; ×120. Fig. 15, later stage showing massive secondary suspensor of embryonal tubes entangled with ends of prosuspensor cells (*ps*); ×70. Fig. 16, 2 embryo systems under conditions of general cleavage polyembryony, with many embryos on ends of isolated prosuspensor cells; ×70. Fig. 16*a*, prosuspensor cells belonging to fig. 16, detached during dissection; larger embryo probably one of several derived from terminal binucleate cells; ×70. Fig. 16*b*, enlarged view of one terminal embryo showing apical cell in optical section; ×140.

some of these, and the embryos of figures 11 and 12 seem to conform to such an origin. Occasional rosette cells may have failed to elongate, but none were found in several embryos corresponding in stage to that of figure 7.

PODOCARPUS MACROPHYLLUS MAKI.—The species labeled *P. chinensis* in the conservatory of the New York Botanical Garden fits PILGER's description (**19**) of the subspecies *maki* (Seib.) very closely. It is rather certain that the plants in Darlington, North Carolina, which furnished the embryological material for COKER's investigation belong here. As PILGER considers *P. chinensis* a synonym under *P. macrophyllus maki*, the latter name is used here.

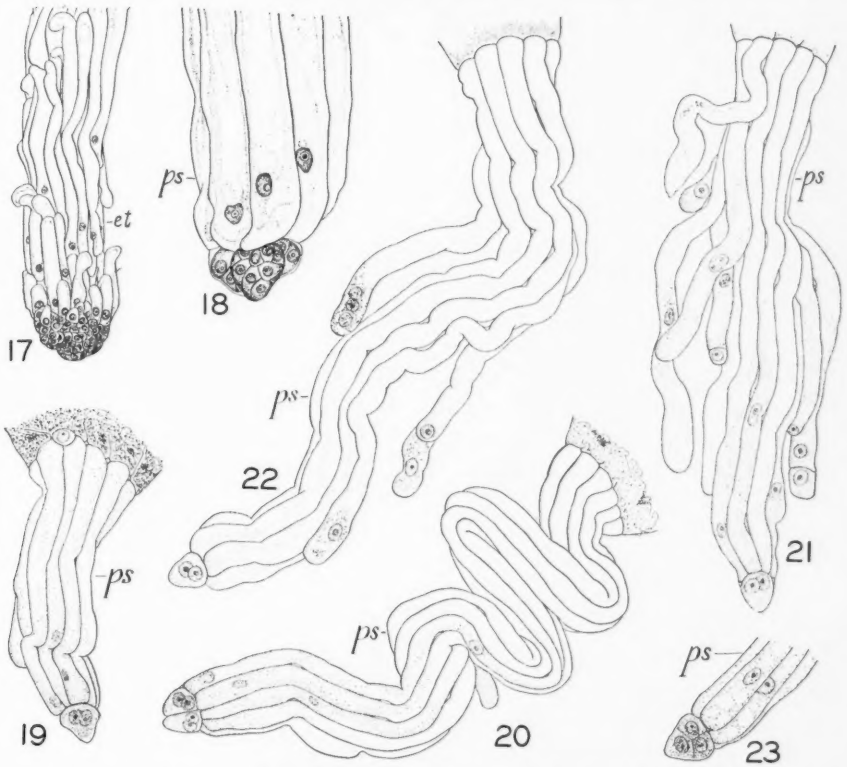
A few stages in the proembryo of this species were described by COKER (**8**), who reported sixteen free nuclei before cell walls appear. He shows two figures with the prosuspensor beginning to elongate, both of which would precede figure 19.

Recently TAHARA (**26**) described the proembryo of *P. macrophyllus* from material in Japan. He states that the proembryo forms walls after sixteen free nuclei have been formed, and his account agrees in its essentials with that of COKER (**8**) for this species. TAHARA shows definitely that in another species, *P. nagi*, the binucleate cells are so organized from the mitosis of a uninucleate walled cell of the proembryo. He shows also in his textfigure 4 that two binucleate cells may be formed occasionally in the proembryo of *P. macrophyllus*. Thus there is no occasion for assuming that a single binucleate cell ever splits longitudinally to form two units, where these are observed in later stages.

Figure 19 shows a deposit forming above the prosuspensor, faintly coral-like, in a stage of formation of the callose plug described by COKER (**8**). This deposit is also shown in figure 20, but is not shown in most of my other preparations because these plugs had been removed during dissection in a search for rosette cells. Figure 19 shows a single isolated rosette cell, and a few similar rosette cells were found in other embryo systems, but a tier of rosette cells is absent. COKER (**8**) speaks of a "rosette," but refers to the free nuclei situated above the prosuspensor, which disintegrate when the callose plug is formed. Most investigators on conifer embryogeny have used the term rosette for a tier of walled cells in the original sense of MIRBEL and SPACH (**18**), but of course COKER'S rosette serves to designate its position, if one were present.

Figure 20 shows two binucleate cells at the tip. These may have had this organization in the proembryo. Usually only a single binucleate embryonic cell is found (figs. 19, 21, 22), and when this cell passes into the next stage it forms a 4-celled embryo with the cells arranged in tetrad form (fig. 23). The mitotic figures of this division were not actually observed in *P. macrophyllus maki* but were in *P. totarra*. A number of the 4-celled stages similar to that of figure 23 could be found, but it happens that none were observed in later stages with double cell masses that might have come from the two terminal cells shown in figure 20.

Figure 21 shows eight of the fourteen cells of the prosuspensor separated from the terminal binucleate cell, so that only six are left in contact with it. Two of these cells have already formed at their ends embryos which resemble ones with primary suspensors. In figure 22 one of the prosuspensor cells has three nuclei without walls between, and another has an elongated cell in place of a short em-



FIGS. 17–23.—Figs. 17, 18, *Podocarpus urbanii:* Fig. 17, terminal embryo slightly 2-lobed on massive secondary suspensor; ×70. Fig. 18, terminal group of 3 embryos, each with about 16 cells; ×140. Figs. 19–23, *P. macrophyllus maki:* Fig. 19, earliest stage with single rosette cell, prosuspensor bearing single terminal cell. Fig. 20, prosuspensor bearing 2 terminal binucleate cells; ×140. Figs. 21, 22, detached prosuspensor cells forming embryos. Fig. 23, embryonic tip now 4-celled; ×140.

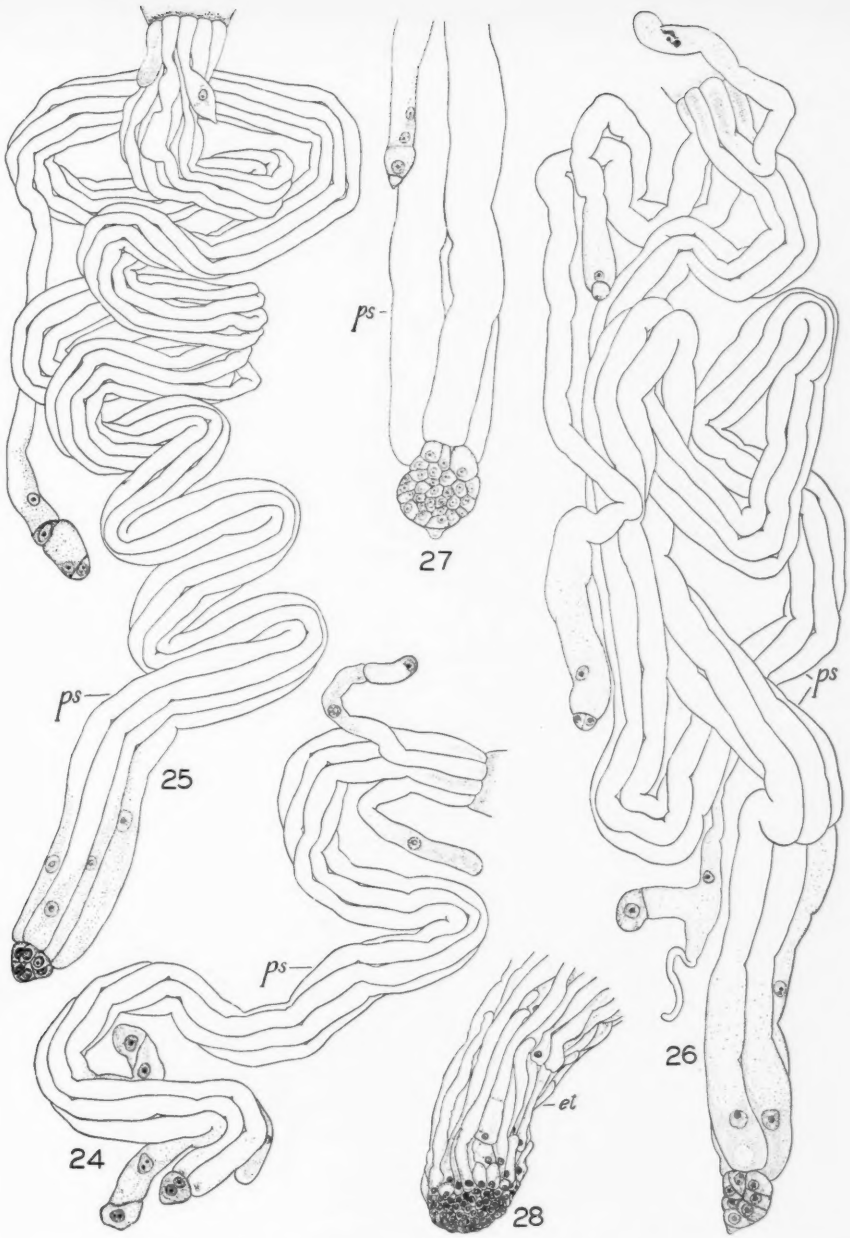
bryonic cell, indicating possible variability in the manner of forming the embryos on the ends of prosuspensor cells. Usually such an embryo does not become binucleate, and—as in the case of *P. urbanii*—the resulting embryos often become abnormal in appearance. Figures 25–27 not only show variations in these secondary embryos but also indicate that they may not be expected to be successful in competition with the larger embryo situated on the end of the prosuspensor.

Figure 25 shows a separated prosuspensor tube which bears two secondary embryos on its end and shows also some very short prosuspensor cells in the rosette region. The small embryo borne on the prosuspensor with an "appendix" in figure 26 is especially interesting. This prosuspensor cell did not elongate fully and the peripheral tubes completely surrounded it for a time while it developed a tapering end. In the twisting of the prosuspensor and the breaking away of peripheral tubes, this cell developed a tube leading to the surface, on the exposed end of which an embryo was formed. Later this tube became liberated, and may have been further loosened during dissection to give the appearance shown in this figure. TAHARA (**26**) observed a similar breaking away of cells of the prosuspensor on the ends of which embryos had been formed.

Several stages in the terminal embryo are shown in figures 23, 25, 26, 27. The persistence in these figures of the pointed end found in the earlier binucleate stage gives assurance that a single terminal embryo initial usually does not split into several smaller units.

While the stage shown in figure 27 is still far from differentiating the meristems of its members, there is no doubt that this mass of cells will contribute to the formation of a single embryo. The more mature stages of *P. macrophyllus* are shown by STILES (**23**). In the cases with single binucleate cells, simple polyembryony is the rule only so far as the terminal embryo is concerned. However, the occurrence of two binucleate cells (fig. 20) would provide exceptions to this rule, and the potentiality of embryo formation in other cells is demonstrated by any cell of the prosuspensor which becomes isolated from the others. The occurrence of these secondary embryos therefore results in a general condition of determinate cleavage polyembryony for *P. macrophyllus maki*.

PODOCARPUS TOTARRA.—This species illustrates another example of the Eupodocarpus type of embryogeny. The proembryo of this species, including *P. nivalis*, was described by SINNOTT (**22**) as having sixteen free nuclei, which become arranged in three tiers with walls forming before another division takes place. The terminal embryonic unit is usually a single binucleate cell, above which are 7–9 prosuspensor cells instead of 14; and the remaining nuclei, which must therefore be less than the number of prosuspensor cells, form a tier of relict nuclei above the prosuspensor tier and soon disintegrate. As in the preceding species, there is no tier of walled rosette cells, neither were isolated rosette cells observed. It appears from SINNOTT'S description of the proembryo, supported by the fact that 7–10, usually 8, prosuspensor cells are observed, that the relict nuclei situated above the prosuspensor must be few, unless the number of free nuclei formed is not strictly sixteen. In any event the proembryo of *P. totarra* seems to differ somewhat from that of *P. macrophyllus maki* (**8**), a difference which is reflected in the smaller number of cells observable.



FIGS. 24–28.—Figs. 24–27, *Podocarpus macrophyllus maki:* Successive stages, with terminal embryo 8-celled in fig. 25; 16-celled in fig. 26; about 60-celled in fig. 27; ×140. Fig. 28, multicellular terminal embryo of *P. urbanii;* ×70.

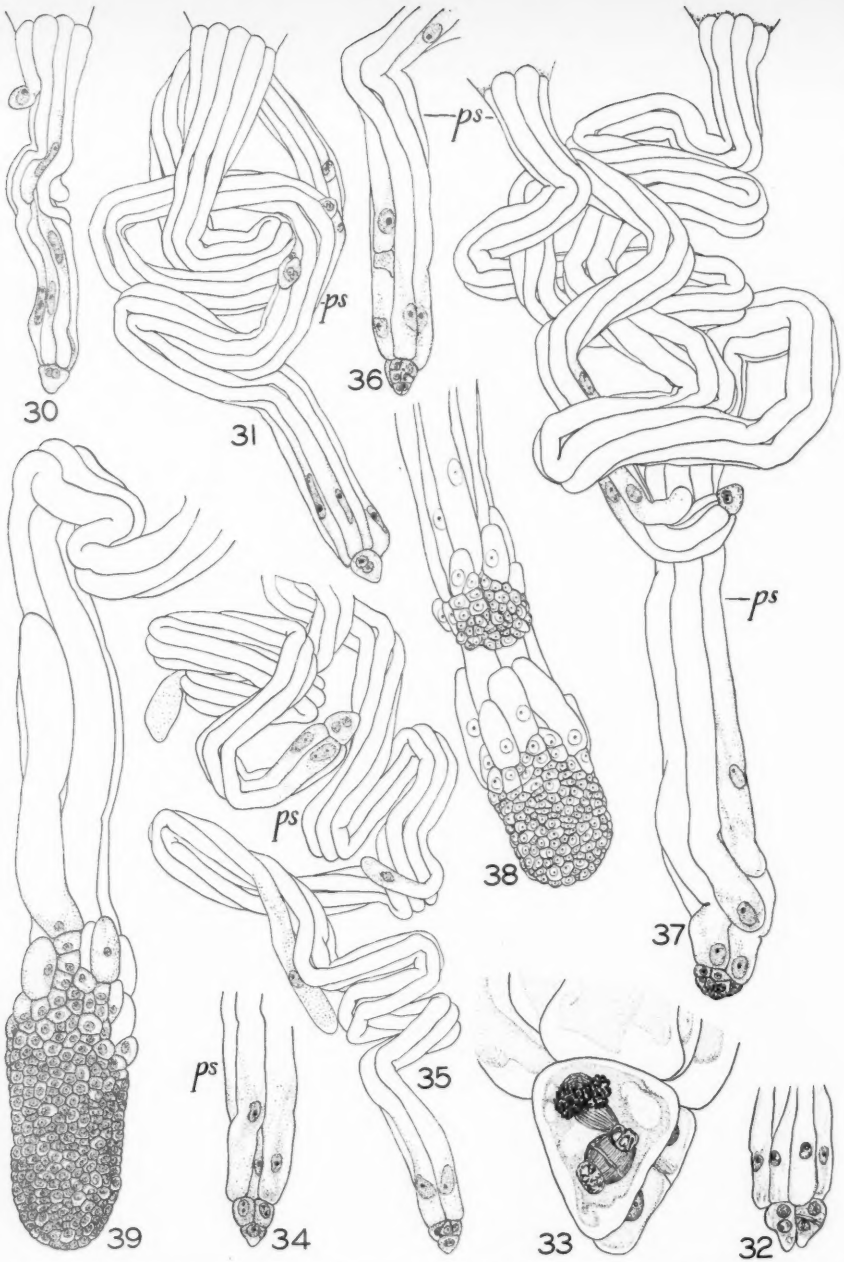
Figure 29 shows the entire section of an ovule of *P. totarra* containing an embryo which is in the stage of figure 31. With the exception of the embryo, the drawing was made from a section of the ovule. The embryo was drawn from a dissected preparation of the same stage in order to show more effectively the coiled prosuspensor. The ovule gives the appearance of having a double integument, but the outer fleshy layer is the epimatium or equivalent of the ovuliferous scale, which—as GIBBS (**12**) and SINNOTT (**22**) have shown—is fused with and completely surrounds the ovule. Figure 29 corresponds closely to SINNOTT's photomicrograph (pl. VI, fig. 7). SINNOTT (**22**) has given the comparative details in the vascular anatomy of the ovules of the Podocarpaceae for a considerable number of genera and species.

Figure 30 is a stage in *P. totarra* which follows the oldest stage of an early embryo illustrated by SINNOTT (**22**, pl. VIII, fig. 32). The latter shows a few relict nuclei above the prosuspensor, but in figure 30 such nuclei could no longer be found. A number of the prosuspensor cells have already broken away. Figures 31, 35, and 37 show successive stages in the coiling of the prosuspensor. In figures 35 and 37, two embryo systems are included. Although the prosuspensor tubes break away as they do in the preceding species, little was found to indicate that they actually form secondary embryos. An earlier stage than figure 30 was dissected from an ovule of *P. hallii* (fig. 40). This embryo also has a single binucleate cell at the tip and is practically the same as *P. totarra*.

Occasionally two terminal binucleate cells were observed, even in stages similar to those shown in figures 30 and 31. Figure 34 shows the terminal embryo when it has become 4-celled. Figure 32 shows the end of an embryo system which had two terminal binucleate cells, one of which remains in this condition and the other has divided to form four cells. In this instance the four cells are not in tetrad form, so that only two of them could be shown in the drawing. Figure 33 is also a system with a double tip. The embryo in the background, of which only the edge is shown, is 4-celled, while the one in the foreground shows each nucleus of the binucleate cell in mitosis. The lower mitotic spindle is in telophase and shows the line of a new cell wall which is forming on the spindle between these two nuclei. The other spindle is in late anaphase with the axis of its spindle foreshortened. Both spindles have an abundance of spindle fibers passing between the newly formed nuclei, but the phragmoplastic spindle fibers that persisted from the previous mitosis (shown in some of the binucleate cells, such as in figure 6*a*) are still to be seen between these two newer spindles.

No doubt the wall between two binucleate cells is delayed until after this pair of spindles has given rise to four nuclei. The 4-celled stage in the terminal embryo is therefore derived directly from the single binucleate cell. It is probable that in all podocarps the binucleate cells directly form similar 4-celled groups. Figures 34

FIG. 29.—Section of entire ovule of *Podocarpus totarra*. Embryo system as found in dissected preparation drawn in place of incomplete embryo system. So-called ovuliferous scale is outer integument which envelops ovule; ×33.



FIGS. 30–39.—*Podocarpus totarra:* Fig. 30, early stage with single binucleate cell borne on prosuspensor; also several cells detached from prosuspensor; ×120. Fig. 31, later stage after great elongation of prosuspensor; ×120. Fig. 32, tip of prosuspensor bearing 2 embryonic units, 1 still binucleate, other 4-celled, only 2 cells showing; ×140. Fig. 33, highly magnified double tip showing 2 division spindles as both nuclei of binucleate cell form 4-celled embryo. Note older phragmoplastic fibers extending between both spindles. Edge of 4-celled embryo shown below; ×650. Fig. 34, 4-celled embryo derived from binucleate cell; ×120. Fig. 35, same; ×120. Figs. 36, 37, 8-celled and 12- to 16-celled embryos; ×120. Figs. 38, 39, later stages forming massive secondary suspensors; ×120.

and 35 show 4-celled terminal embryos; figure 36 has an 8-celled embryo; and figure 37 a 12- to 16-celled embryo. In these early stages of cell formation the total size of the embryo does not increase greatly until many cells have been formed.

The stages between figure 37 and the embryos of figures 38 and 39 were not available for study. Figure 38 shows the terminal embryo separated from a smaller cell mass by its secondary suspensor (*et*). This condition was observed several times. Figure 39 is one of the oldest embryos dissected and shows in optical section that the organization of the plerome of the root tip is just beginning. Except for the cases in which two binucleate cells are found on the end of the prosuspensor, the embryogeny of *P. totarra* usually follows a program of simple polyembryony. The proembryo seems to have undergone some kind of reduction from the type of development shown in other species of *Podocarpus*, and the later stages appear to have become modified so that cleavage polyembryony has become more or less suppressed.

PODOCARPUS GLOMERATUS.—The embryo of this South American species was examined from herbarium material only. The writer examined an undated specimen collected by A. Rimbach (626) "in the valley of the Chimbo river near Guaranda, alt. 2800 m. on the slopes of the western Cordillera" of Ecuador (Field Museum). The embryo complex dissected from this specimen included two systems, the smaller of which was incomplete. The larger prosuspensor in this complex had about twelve cells in its upper part, where it joined the base of the archegonium, and of these about nine extended throughout the entire length to the embryonic tip. The latter was composed of three separate and distinct but closely grouped embryos, which if drawn would give a figure similar to figure 18. The three embryos had all advanced to the 8-celled stage, but they retained the original contours of the three binucleate cells from which each was derived, so that it was evident that three existed in an earlier stage (such as that shown in figure 7*b*). The embryogeny of *P. glomeratus* may be presumed to be very similar to that of *P. urbanii*.

PODOCARPUS CORIACEUS.—The only stages observed with early developing embryos came from a herbarium specimen collected by H. Pittier (8298) in Venezuela, December 26, 1929—"Altos de Galipan, Cerros del Avila, alt. 1600 m." Three embryo systems on relatively short and untwisted prosuspensors were found in the only ovule examined. Their respective embryonic tips were composed of three, three, and two embryonic units, some of which appeared to be past the binucleate stage. The prosuspensors at the top or archegonial ends had about 11–12 cells. Provisionally one would describe the general embryogeny as resembling that of *P. urbanii* more closely than that of any other type which has been described.

PODOCARPUS PURDEANUS.—Two ovules were examined from herbarium material collected by Britton and Hollick (2798) in Jamaica, April 6–7, 1908. One ovule

contained a complex of two embryo systems. The two prosuspensors had 11–12 cells at the archegonial end. The number of binucleate cells or units borne on the end of the prosuspensors could not be determined. The cells of the prosuspensor had become separated, showing a condition similar to that in figure 16, with the terminal embryo larger than that shown in figure 17. The partially disintegrated remains of thirty-three or more small embryos could be observed among the embryo complex of prosuspensor cells.

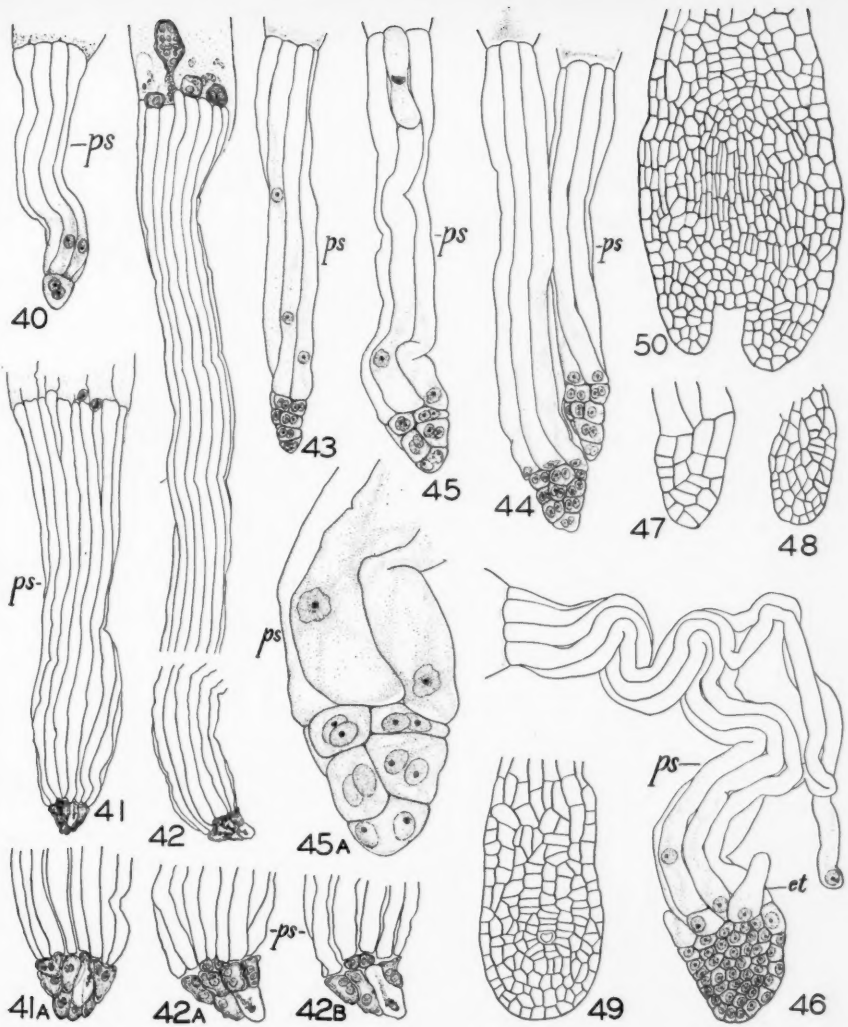
The other ovule had only a single embryo system, with the terminal embryo at about the same stage or slightly larger. The remains of about fourteen small embryos could be counted in and around the tangle of separated strands of the prosuspensor.

PODOCARPUS MATUDAI.—Only a single ovule of this Mexican species was examined. This came from the Matuda collection (956), Mt. Avado, somewhere in Chiapas, the extreme southern state of Mexico. The embryo complex contained four embryo systems, each with about twelve prosuspensor cells shown at the top. The lower end of the prosuspensor of the largest system showed nine cells extending to the tip, where the largest embryo had been broken off. However, this largest terminal embryo was lost in dissection. One of the smaller aborted embryo systems had a double tip. In one of these two cells the binucleate condition could be observed distinctly. Another one of the aborted system had an embryonic tip which showed several cells, but the nuclei could not be seen.

All the Central and South American species of *Podocarpus* thus far examined (including several species not specially cited here) had about twelve prosuspensor cells at their archegonial ends. None of them agree with the condition found in the 8-celled prosuspensors of the New Zealand species, *P. totarra*, *P. hallii*, and *P. nivalis*.

PODOCARPUS, SUBGENUS STACHYCARPUS

PODOCARPUS GRACILIOR.—This species is placed in the *Stachycarpus* subgenus by PILGER (**19, 20**). The specimen, which contained a pair of early embryos of great diagnostic value, came from collections of Edgar A. Mearns, expedition to South Africa (382), collected on June 7–8, 1909, "between Aljoro-o-Nyou and Narok river at an altitude 2000 m." One of the embryo systems is illustrated in figure 41, with an enlarged detail of the tip shown in figure 41*a*. There are about twenty cells in the prosuspensor, which bears ten cells at its tip, and in most of these cells the two nuclei could be recognized under oil immersion. This remarkable embryonic type was observed and reported in a slightly earlier stage for *P. usambarensis* (**4**). The embryo of figure 41 has a cell in which the protoplasm has collapsed and the cell is nearly empty. There is a cell in the interior completely surrounded by the others. No later stages of *P. gracilior* were obtained, but four embryos of *P. amarus* belonging to this taxonomic category were obtained from



FIGS. 40–50.—Fig. 40, early embryo of *Podocarpus hallii;* ×120. Fig. 41, same of *P. gracilior* with 18–22 prosuspensor cells, bearing about 10 binucleate cells below; ×80. Fig. 41*a*, enlarged view of lower end of fig. 41; ×155. Fig. 42, early embryo of *P. nankoensis*. Prosuspensor broken in dissection, but nearly all is represented. It is made up of 18–20 cells, with a few isolated rosette cells above; it has also a club-shaped ball of cytoplasm showing 11 nuclei that may have come from relict nuclei of egg cavity above prosuspensor, shrunken thus during drying; ×80. Figs. 41, 42, from dried herbarium material. Fig. 42*a*, *b*, two views, tip of fig. 42, in different planes of focus; ×155. Figs. 43–50, *Phyllocladus alpina:* Fig. 43, earliest stage, 5 prosuspensor cells, bearing 10 binucleate cells below; ×110. Fig. 44, 6 prosuspensor cells at left and 5 at right (*ps*), each bearing 11 binucleate cells; ×110. Fig. 45, 5 prosuspensors bearing 9 binucleate cells; ×110. Fig. 45*a*, tip of fig. 45 showing cytoplasmic strands between nuclei of terminal cell; ×250. Fig. 46, oldest stage dissected. Figs. 47–50, successive stages from sections.

the A.D.E. Elmer collection of plants of the Philippine Islands (11862). This specimen was collected in September, 1909, on the island of Mindanao, Todaya (Mt. Apo), District of Davao.

PODOCARPUS AMARUS.—Three ovules were dissected. One endosperm contained a complex of two embryo systems. The prosuspensor of each system showed that it was composed of about twenty elongated cells at the upper or archegonial end, and it had elongated to a length of 7–8 mm. On the lower end of one of these systems, three embryos were attached by their secondary suspensors. The terminal and largest embryo had a secondary suspensor nearly 2 mm. long and was a conical mass of embryonic tissue of seventy or more cells. The next embryo, well separated but closely behind the first, was only slightly smaller, and the third was composed of about fifty cells. The secondary suspensors of all three were closely interwoven distally, and near their junction to the prosuspensor 7–8 embryos were found which were much smaller and had not developed secondary suspensors. Nearly all these 10–11 embryos were situated so that they seemed to belong to the same system.

There were six separate embryos grouped near the end of the other prosuspensor. The largest of these had about twenty-five cells and was just beginning to form its secondary suspensor. Very few nuclei could be seen in cells of the prosuspensor, but the entire complex was covered with many starch-filled endosperm cells. It appeared that a few small rosette embryos were present, but this region was covered with a deeply stained basal plate deposit which obscured these details.

Both of the other two ovules of *P. amarus* whose endosperm was dissected contained single embryo systems; both showed about twenty cells in the upper ends of their prosuspensors, and in one of them there was a small rosette embryo, probably derived from an isolated rosette cell. One of these systems had elongated to about 8 mm. and was tipped by a group of six separate embryos, the largest of which was beginning to form its secondary suspensor and was composed of about thirty-five cells. The other embryos of this group were smaller, and a few of them were somewhat irregular in shape. About 1 mm. back from the tip was the largest embryo, composed of about forty-five cells but without a secondary suspensor. There were a few other very small embryos, some of which appeared to have been formed on the ends of some of the prosuspensor cells.

The other system was 9 mm. long but bore four terminal embryos on secondary suspensors. These embryos had slightly unequal, entangled secondary suspensors; the largest had about eighty cells, the others were estimated at sixty-five, fifty, and forty cells each. About 0.5 mm. back of this group was an embryo of about twelve cells and several smaller embryos of only a few cells, some of which appeared to be so situated that they may have been derived from the ends of prosuspensor cells. Some of the prosuspensor cells had formed internal chains of embryonic cells.

If one may assume that the embryos of *P. usambarensis*, *P. gracilior*, and *P. amarus* are all essentially similar and present a general outline of the type of embryogeny in this group, the conclusion is that these have cleavage polyembryony. Each of the binucleate cells at the end of a prosuspensor cell in *P. usambarensis* and *P. gracilior* gives rise to a separate embryo, unless it becomes aborted in an early stage of development. The numerous larger embryos in *P. amarus* must have been derived from this number of binucleate cells.

Protopodocarpus, Section Nageia

The embryogeny of *Podocarpus nagi* has been investigated recently by Tahara (**26**), who described the proembryo as having thirty-two free nuclei before walls are formed. Seven to nine of these nuclei become surrounded by walls, forming a tier of cells in the lower end of the archegonium. Above this tier the remaining nuclei become organized into another tier, with walls between the nuclei but remaining open to the egg cavity above. The next division of the nuclei forms a tier of 23–25 prosuspensor cells with an open tier of relict nuclei above. No rosette is formed and no isolated rosette cells were observed. The nuclei of the lower tier divide to form embryo initials which remain binucleate for the early period of elongation of the prosuspensor. In this account Tahara has answered the question of the precise origin of the two nuclei of the binucleate cells. His series of figures is complete enough to demonstrate that the two nuclei in a binucleate cell are not some of the thirty-two free nuclei held over but all originate from a cell with a single nucleus. This provides the basis for assuming that all binucleate cells in the Podocarpaceae have a similar origin.

The embryogeny of the Nageia group is very similar to that of *P. usambarensis*, *P. gracilior*, and *P. amarus*. An early embryo of *P. nankoensis* is shown in figure 42. Here the prosuspensor, bearing a group of embryonic cells, is in an early stage of development. This embryo system, broken during dissection, has only a negligible portion of the prosuspensor missing. There are 19–20 prosuspensor cells in its upper part, with several rosette cells; at the embryonic end at least eighteen could be counted. The embryonic tip is composed of a group of 10–11 binucleate cells shown in surface view in figure 42*a*, and in a median plane of focus in figure 42*b*, where only a few cells remain hidden. One of the largest cells, in a central position, has become abortive and lost nearly all of its protoplasmic content. Two very small cells are situated in the interior, completely surrounded by prosuspensor cells above and by neighboring binucleate cells below. Owing to shrinkage, they show through in the surface view; in living condition they would be covered on all sides. It is only with respect to these small cells that this embryo differs from that of *P. gracilior* (fig. 41) and of *P. usambarensis*.

Three embryo systems were observed in this stage. One of the embryos of *P.*

nankoensis, not illustrated, had no internal binucleate cell and another appeared to have only one such cell, which was completely surrounded. Both of these unillustrated embryos were without the abortive cell shown in figures 41 and 42.

These variations may indicate that the exact arrangement of the cells and the stage of abortion of some of them are variable and may be relatively unimportant. The rosette region at the top of the prosuspensor in figure 42 appears to have four rosette embryos. One of them extends upward and has about twelve nuclei in a dense mass of cytoplasm, surrounded by a common wall but with no walls separating them. This may not be a true embryo but an appearance due to some kind of artifact formed during drying of the specimen, in which the free nuclei in the egg cavity above the prosuspensor were collected into a ball of cytoplasm. One rosette embryo is 2-celled, two others remain single-celled. Rosette cells similar to the latter were observed in the embryo of *P. usambarensis* (**4**). There were rosette cells and embryos similar to the smaller ones in figure 42 on one of the other two embryo systems not illustrated.

While the embryos of *P. nankoensis*, *P. nagi*, and *P. blumei* which were dissected from herbarium specimens in later stages of development could be described and would provide all the evidence needed to conclude that cleavage polyembryony is found regularly in the Nageia group, it is deemed unnecessary to retain this portion of my description. The recent publication by TAHARA has shown this clearly from material properly killed and fixed. TAHARA'S textfigure 12 shows the general condition, after the embryos derived from the binucleate cells have become separated on their own secondary suspensor. My material confirms his observations in all essentials; there is no doubt that cleavage polyembryony is the normal condition in the Nageia group.

The only difference in the observations of the writer made upon herbarium material indicates a smaller number of cells in the prosuspensor and a few more embryo initial cells in the terminal group. Occasionally one or two of the cells in the terminal group may be situated internally and completely surrounded by others, as shown in figure 42, 42*a*. TAHARA'S figures, however, indicate that there may be more than nine; his textfigure 10*a*, showing parts of five cells in the lower tier in the same longitudinal section, may have more than nine, and his textfigure 10*b* shows a cell centrally placed so that it may be completely surrounded by others, as shown in figure 42, 42*a*.

The later embryo systems of the Nageia group, in which the surviving terminal embryos had become large and developed two cotyledons, usually failed to show more than a few of the remaining abortive embryos. With very careful dissection, it was usually possible to count the upper ends of the prosuspensors so that the number of embryo systems could be determined.

In one ovule five embryo systems were found with the remains of more than fifty

small aborted embryos; an ovule with six embryo systems had more than forty aborted embryos; another with four systems had more than twelve. *P. blumei* appeared to have similar conditions. In all the species of the Nageia group there were two cotyledons, but one out of three of the embryos of *P. blumei* had three.

Embryogeny of Phyllocladus

The first account of the special morphology of *Phyllocladus alpinus* was given by Kildahl (**14**). This was incomplete but was amplified considerably by Young (**27**), who confined her account to the stages through fertilization. Kildahl had included a few stages in the proembryo up to the stage with eight free nuclei, with no indication that wall formation was imminent at this stage. It is likely, therefore, that sixteen free nuclei are followed by wall formation. Since the earliest embryos available had 5–6 cells in the prosuspensors, bearing 9–11 binucleate embryonic cells, the events are probably as follows: sixteen free nuclei are organized with walls arranged more or less in tiers of 5, 4, 3, 3, 1 or 6, 4, 3, 2, 1 cells, with the upper 5- or 6-celled tier still open above. This is followed by a nuclear division in each cell, the upper tier giving rise to 5–6 prosuspensor cells and an equal number of relict nuclei (which soon go to pieces) in a tier without walls. The 9–11 walled cells below the prosuspensor tier would thus each become binucleate, and—following elongation of the prosuspensor—an embryo similar to figure 43 would result. This is in general agreement with Looby and Doyle's account of the proembryo of *Saxegothaea* (**10, 17**), which differs only in having a shorter prosuspensor made up of a smaller number of cells.

Holloway (**13**) in his account of the embryogeny of *Phyllocladus alpinus* seems to have overlooked the binucleate condition in the early embryonic cells. Figure 43 represents one of the earliest stages obtainable of the embryo of *P. alpinus*. This example has five cells in its prosuspensor and bears ten binucleate cells below in four tiers of 4, 3, 2, and 1 cells each. Figure 44 shows two adjacent embryo systems. The larger, at the left, has six prosuspensor cells and 10–11 binucleate embryonic cells in four tiers, and the one at the right with five cells in its prosuspensor has ten embryonic cells, nine of them binucleate and two of them definitely uninucleate. Figure 45 has five cells in its prosuspensors (or six counting the short one above) and 9–10 binucleate cells below them. The enlarged drawing of this tip (fig. 45*a*) shows the telophasic spindle fibers passing between the two nuclei, as in several of the cells in *Podocarpus dacrydioides* (fig. 6*a*).

Figure 46 shows the oldest embryo obtained from dissected material. In this at least six cells are found in the prosuspensor. The embryo has become large and multicellular, and the beginning of elongation of the embryonal tubes to form the secondary suspensor is shown. Although *Phyllocladus* has a very short prosuspensor, this member appears to be relatively longer than that of *Saxegothaea* (**10**).

Several stages in the embryogeny of *Phyllocladus alpinus* were also studied from serial sections. Four of these are shown in figures 47–50. Figure 47 is a stage just after the embryonic cells have all become uninucleate, and would follow one of the embryos shown in the dissected stages such as figure 44 or 45. It is probable that each of the binucleate cells forms four uninucleate cells at a single step, as was shown in figure 33. This, accompanied by a slight enlargement, would result as shown in figure 47, in which all cells were uninucleate. Here the apical cell arrangement with an appearance of segments may be due to an origin of groups of four uninucleate cells from each of 9–11 binucleate cells arranged in a conical mass. In any event the mass shown in figure 47 did not come from a single apical initial cell, and an apical cell need not be present to give this appearance of segmentation.

The next stage (fig. 48) differs little from that of figure 47 in its total size. There are, however, more than twice as many cells. An apical initial cell was not clearly recognizable in this stage. Figure 49 is a median section in a later stage in which the cells are larger, as well as more numerous, and the organization of the pleromic apex of the root is indicated by the centrally placed cell shown with a nucleus. All parts of this figure (about two-thirds of it) situated above this cell belong to the calyptroperiblem (**6**), which merges into the secondary suspensor, while the embryo proper is derived from the cells below this pleromic apex. If a circular line were drawn to include the lower parts of this figure and the cell with the nucleus included on its circumference, all the cells which give rise to the essential parts of the later embryo except the calyptroperiblem would be included.

Figure 50 is a section through the later embryo after the cotyledons have appeared, but it is not a perfect median section, so that it shows only imperfectly the region of the pleromic apex of the root tip, which is now situated about three-fourths of the distance from the lower end of the figure. Two cotyledons are shown, and (on the left side) a series of narrow elongated cells mark the edge of the plerome in the hypocotyl region. The stem tip does not appear to be elevated at this stage. This is the largest embryo which was available for study. One stage shown by HOLLOWAY (**13**) has the same width and differs only in being somewhat longer throughout.

Discussion

Before proceeding with the discussion, it is desirable to correct some errors and misinterpretations which have appeared in certain accounts. I have also fallen into an error in my interpretation of the embryogeny of *P. macrophyllus maki*—given after COKER (**8**) under the name *P. coriacea*. The diagram published elsewhere (**1**, fig. 18) is incorrect. No primary suspensors are formed by the terminal embryos borne on the end of the prosuspensor. The small embryos that appeared on the ends of single suspensor elements were erroneously interpreted as borne on

primary suspensors. I know now that the primary suspensor is not usually present in any species of *Podocarpus*, but there are secondary embryos formed on the ends of detached cells of the prosuspensor, and these might resemble embryos borne on primary suspensors.

The problem of an apical cell in *Phyllocladus* is somewhat related to the binucleate cells. It may be assumed from the observations on *P. totarra* (fig. 33) that each binucleate cell gives rise to a group of 4-walled cells at one step. After a similar division, the embryo of *Phyllocladus* would have 40–44 cells grouped in 10–11 areas. These might appear as the segments resulting from apical cell growth. It is easily possible, therefore, especially where the shape of the entire embryonic mass of cells is conical, to find a terminal group of four tetrahedral cells that give the appearance of an apical cell which has produced a single-celled and 2-celled segment next to it, with the other 4-celled groups assuming the appearance of older segments. Although I illustrated such an embryo (**1**, fig. 20*D*), pointing out that only the apical cell comes from the lowest binucleate embryonic initial and cannot be responsible for growth of all parts of the embryo, I am convinced now that the existence of a true apical initial cell in *Phyllocladus* is very doubtful; if present at all, it cuts off only a small number of segments before it disappears.

In regard to HOLLOWAY'S error (**13**) in failing to observe the binucleate cells in the early embryo of *Phyllocladus*, it should be stated in justice to him that it is very difficult to recognize this condition in serial sections alone; it might have been overlooked in the sections left from YOUNG'S investigation (**27**), which I have before me.

The differences in embryogeny within the genus *Podocarpus* are remarkable. Extremes are found in the number of prosuspensor cells, from about 19–22 in *P. usambarensis* (**4**) to seven, eight, or nine in *P. totarra* and *P. hallii*. If the entire family is considered this variation is still greater, since the number of cells in the prosuspensor of *Phyllocladus* drops to five; and in *Saxegothaea*, according to DOYLE and LOOBY (**10**), some embryos have four and others only three. Likewise the variation in the length of the prosuspensor is extreme. The longest known are found in the plants closely related to *P. usambarensis*, and in the Nageia group, the ones having twenty or more cells in the prosuspensor. For example, PILGER (**20**) mentions that he found a suspensor system in *P. amarus* which was 30 mm. long. Of course this doubtless included a considerable portion of secondary suspensor, even though PILGER mentioned that the embryo at the end was still very immature. Within the genus *Podocarpus* the prosuspensor is usually relatively long, but in some other genera, such as *Phyllocladus* and *Saxegothaea* (**10**), the suspensor is very short. In one of these two genera it reaches its known limit for shortness. There is also considerable variability in the behavior of the cells of the prosuspensor. They may remain closely attached to the terminal cell or group of

cells as in *Podocarpus spicatus* (4) and in *Dacrydium* (3), or they may become separated so that all or nearly all become embryonic as in *P. urbanii*.

The presence of 1–12 or more binucleate cells at the tip of the prosuspensor is another remarkable feature. Thus far, all podocarps examined in the proper stage have had binucleate embryonic cells. This list now numbers nineteen species when *Saxegothaea* is included, and embodies four genera of the family. Unfortunately SAHNI (21) makes no mention of the embryonic cells in his detailed study of *Acmopyle*, but his figures show that it has a long, well-developed prosuspensor, and his work proves on other grounds that this genus belongs to the Podocarpaceae. Likewise LAWSON (16) has overlooked this condition in *Microcachrys*, if it occurs there. His investigation, however, like that of HOLLOWAY (13) on *Phyllocladus*, was confined to the study of serial sections.

In spite of the variations within the family in the relative length and number of cells of the prosuspensor and in the number and arrangement of the binucleate embryonic cells, there is an orderly transition from one extreme to the other, which is not in great disagreement with the arrangement of genera, subgenera, and species. Some transfers in the taxonomic groupings within the genus *Podocarpus* will be necessary, but these will be discussed later.

If the primary comparisons could be made between the proembryos, this no doubt would afford the most satisfactory basis for the larger groupings. However, the proembryos of only five types have actually been observed. These are *Saxegothaea* (10, 17), *Podocarpus spicatus* (22), *P. macrophyllus maki* (8, 26), *P. nagi* (26), and *P. totarra* (22). With these as a background, a careful study of the early embryos of the remaining species makes it possible to estimate the probable proembryos in most of the remaining forms examined. This was done for *Phyllocladus alpinus* in an earlier part of this paper. It can also be done for *Dacrydium*.

Dacrydium has 9–11 prosuspensor cells, which—if produced in the usual manner—have had an equal number of relict nuclei in a tier above the prosuspensor. If walls are formed in its proembryo after sixteen free nuclei have appeared, and 5–7 cells arranged in two unequal tiers below are to form this number of embryo initial cells, this would leave 9–11 nuclei in a third tier, whose division would result in a prosuspensor tier and a tier of relict nuclei, with the 5–7 embryonic cells becoming binucleate. Variations are to be expected. An occasional nucleus in the upper tier which fails to divide and joins the group of relict nuclei would decrease the number of prosuspensor cells, etc. In the proembryo of *P. dacrydioides* a similar program would be expected, except that here the embryonic cells are fewer and would be arranged in a single tier. *P. usambarensis* and *P. gracilior* have early embryos which are very similar to *P. nagi* as described by TAHARA. All these must have a proembryo in which thirty-two free nuclei are formed before walls appear.

Of course, some of the proembryos of the Podocarpaceae just described are only

hypothetical. Several of those actually observed should be more fully investigated. Comparisons of the proembryos of this family cannot be made on the basis of fully known facts, but comparisons of the early embryos—in which the nearly comparable stages have actually been observed—are possible. Figures 51 and 52 show a series of diagrams, drawn to the same scale of magnification, embracing the types of early embryos found in the Podocarpaceae, so far as these have been investigated. The numbers of binucleate cells in the terminal group are shown by numerals placed below them and the numbers of cells in the prosuspensor by numerals above.

This series of embryos probably includes the extremes of embryonic types to be found in this family. The number of binucleate cells ranges from twelve or more in four tiers (fig. 51) below the prosuspensor, through about 7–11 binucleate cells all arranged in a single tier, down to the single binucleate cell shown at the right (fig. 52). One could not expect to find fewer than one binucleate cell at the tip, or to find fewer than three in the prosuspensor at the other extreme. The prosuspensor varies, not only in the number of cells of which it is composed but also in the extent to which these elongate. In future researches on podocarps, it may be expected that the remaining species will fit somewhere in this series. The order from left to right is in general agreement with the taxonomic arrangement of the genera and species within the family, but it includes two rearrangements in the groupings. *Dacrydium* is inserted near the middle of the series; and the Nageia group, which is placed beside it, must include also *P. usambarensis*, *P. gracilior*, and their close relative, which would be transferred from the subgenus *Stachycarpus*. It is evident that the genus *Podocarpus* breaks up into several smaller groups in a new arrangement, among which *Dacrydium* may be inserted.

The genus *Podocarpus* presents almost as great variety in embryogeny as the entire family Podocarpaceae. While figures 51 and 52 also include the three genera *Saxegothaea*, *Phyllocladus*, and *Dacrydium*, the addition of these does not greatly increase the range of types shown by the species of *Podocarpus*. This genus seems to embrace sufficient variety to deserve segregation into several genera, although such splitting would seem premature in the present state of our knowledge. It would also seem impractical, if the only differences are to be found in embryogeny. No doubt there are external taxonomic differences correlated with these variations in embryogeny. A careful study of the detailed morphology and embryogeny of all possible forms should be made; then the correlated external taxonomic differences will become apparent.

The question as to which embryogeny is the most primitive presents itself. The answer given from a study of proembryos would lead to the selection of a type close to *P. usambarensis* and the Nageia group, since this type would have thirty-two free nuclei before walls are formed, in closer agreement with the number found

in the Araucaraceae (**7, 11**) and *Sciadopitys* (**25**). The same selection is suggested when the series of early embryos is examined. The modification in evolution within the family seems to have taken place in both directions from near the center of the series shown by figures 51 and 52 combined. Whether the embryonic cells are arranged in a single tier or in two is relatively unimportant, since a progressive narrowing of the archegonia would bring about the change shown in the direction of *P. spicatus* and *Saxegothaea*. It is not likely that one would select either of the extremes in this series if the postulated prototype is to have features in common with other groups of conifers.

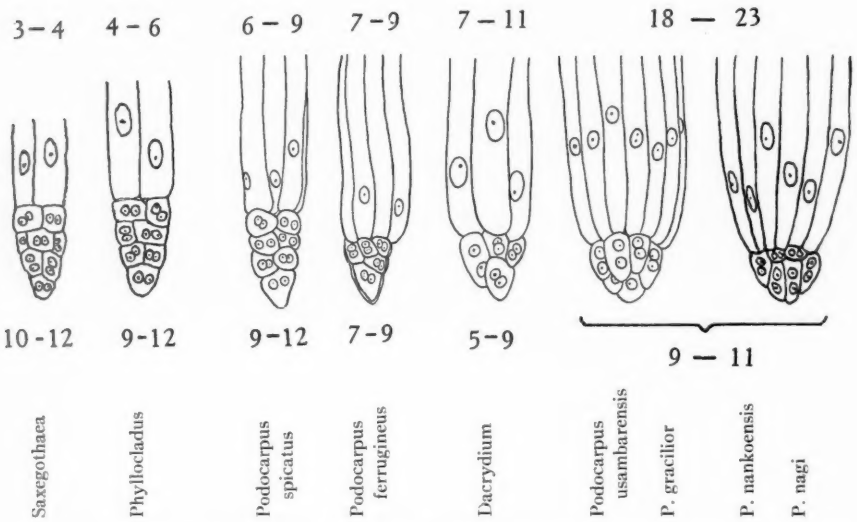


FIG. 51.—Diagrammatic comparison of early embryos of Podocarpaceae showing, by numerals placed above the figures, variations in number of prosuspensor cells and by numerals below the figures, variations in number of binucleate embryonic cells. The latter are arranged in 4, 3, 2 tiers or in a single tier (continued in fig. 52).

The embryogenies of *Araucaria* (**7, 24**) and *Agathis* (**11**) have been unique and puzzling. They could not be related closely to any other conifer, partly because the numbers of free nuclear divisions in the proembryo have greatly exceeded those known to occur in other genera and partly because the organization of the early embryo is unique. It is desirable, therefore, to digress in order to point out a feature wherein the embryogeny of some of the podocarps offers some clues in their similarities.

Figures 41 and 42 of *P. gracilior* and *P. nankoensis* show a plan of cellular organization very similar to the early embryo of *Araucaria* and *Agathis*. As

pointed out, these podocarps and also *P. usambarensis* (4) must have thirty-two free nuclei in their proembryos before walls appear. This is a condition nearer to the araucarians than is found in any other member of the Podocarpaceae. Furthermore, this cellular organization sometimes includes a few internal cells completely surrounded by others. In araucarians the number of cells included in this internal group is much greater than in these podocarps. They are all uninucleate; also the cells that form the cap are more numerous, and these are all uninucleate. The number of cells in the prosuspensor of the Araucariaceae and of these podocarps is about the same, and these are uninucleate in both families. *Araucaria* has simple

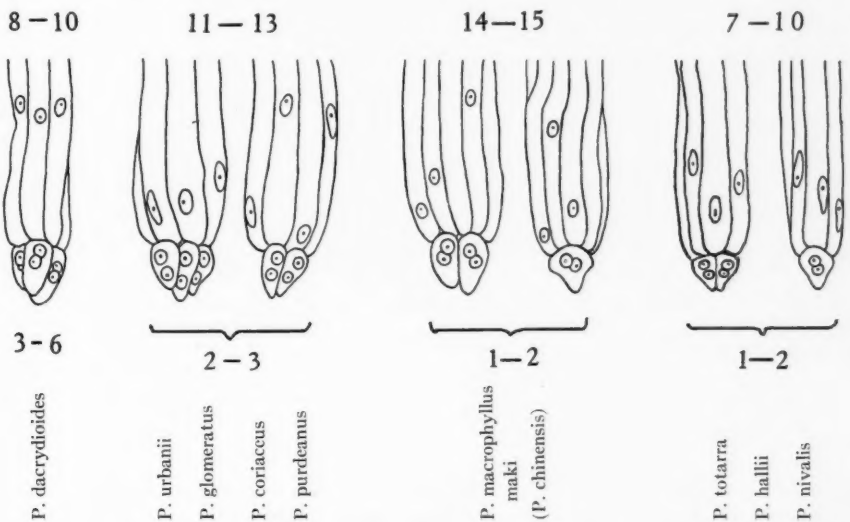


FIG. 52.—Comparison of early embryos of Podocarpaceae (continued). *Podocarpus* includes the widest range of variation found in any genus of Coniferales. In the above group the number of embryonic cells ranges from 6 to 1, all in a single tier. All diagrams are approximately to same scale.

polyembryony while the species of *Podocarpus* in question have cleavage polyembryony. Some of the binucleate cells in these podocarps may abort without forming embryos, while in *Araucaria* and *Agathis* all the peripheral cells that form the cap are abortive, and only the group of internal cells actually forms the embryo. While the embryogenies of araucarians and certain podocarps still stand far apart, these comparisons are the closest that can be made between the Araucariaceae and any other group of conifers. There are, of course, many other morphological similarities between these two families.

The genera and species which appear to have simple polyembryony are *Phyllocladus*, *Podocarpus spicatus*, *P. ferrugineus* (4), and *P. totarra*, together with the

forms closely related to the latter. Those with some degree of cleavage polyembryony are *Saxegothaea* (10), *Dacrydium* (3), *Podocarpus dacrydioides*, *P. imbricatus*, *P. urbanii*, *P. macrophyllus maki*, *P. glomeratus*, and *P. purdeanus*. In a number of these, simple polyembryony may be the apparent condition, for when cleavage polyembryony occurs it may be of the determinate type shown by *P. urbanii* and *Dacrydium* (3). The more open or indeterminate type of cleavage polyembryony occurs in the Nageia group of *Podocarpus*, including with them *P. usambarensis*, *P. gracilior*, and *P. amarus*. All of these forms thus far studied have cleavage polyembryony.

All the Podocarpaceae show a polyembryonic organization by the occurrence of the binucleate cells; those with more than a single binucleate cell are essentially polyembryonic and of the cleavage type. The podocarps appear to be polyembryonically compound. Whether an actual separation of the respective cell progenies of the binucleate embryonic units occurs where several are present makes no essential difference, except in the appearance of the embryonic complex. There may be a closed system in which the cleavage polyembryony does not always show its fundamental nature by separations of many small embryos. For example, the embryo of *Phyllocladus*, which produces only one embryo per fertilized egg, develops in such a manner that the progeny of only one of the terminal binucleate cells is likely to contribute the meristematic parts of the later embryo. The other binucleate cells contribute only to the secondary suspensor and calyptroperiblem. In a strict sense, therefore, a form of determinate cleavage polyembryony is present even in the organization of this embryo, where competition between the parts has been pushed back to a very early stage and is so coordinated that it never comes to expression. Thus the embryogeny of *Phyllocladus* may be described as having simple polyembryony in spite of this polyembryonic organization. The same would be true of *Saxegothaea* in its normal development, in which, however, DOYLE and LOOBY (10, 17) found that some kind of cleavage polyembryony becomes recognizable in about 15 per cent of the embryo systems. As already pointed out, the embryogeny of *P. urbanii* shows cleavage polyembryony for the two or three embryonic binucleate cells. It has also embryos formed on prosuspensor cells as extensively as shown in figure 16 in about 10 per cent of the embryo systems, although the condition shown in figure 9 was found in the majority of the systems dissected from the ovules examined. *P. macrophyllus maki* is similar when two binucleate cells are present, and it agrees with respect to secondary embryos that may be formed on the ends of isolated cells of their prosuspensors.

In *P. totarra*, which usually has a single binucleate embryonic cell, there is normally no cleavage polyembryony from the terminal embryonic portion of the system, and although the prosuspensor cells may break away from the tip as they do in *P. urbanii* and *P. macrophyllus maki*, they do not seem to form embryos

at their tips. The *P. totarra* type has attained simple polyembryony as the usual condition, and only when two binucleate cells are present would it show cleavage polyembryony.

The question as to whether cleavage polyembryony is primitive or advanced may possibly be open to question. DOYLE and LOOBY (**10**) have concluded, possibly rather hastily, that cleavage polyembryony arose from simple polyembryony in the families in which it occurs, and is therefore more advanced. It is both primitive and advanced in the sense that this condition is found throughout the Coniferales, among members of the most primitive as well as the most advanced families. It is found even in the Gnetales. The writer has contended that it may have had a single origin near the beginning of the evolution of Coniferales, and that the change to simple polyembryony occurred in isolated genera or groups of genera. Cleavage polyembryony is so widespread in this order that—out of thirty-five genera in which conditions are known—only nine genera and parts of two others have simple polyembryony. The conditions in eight genera are not known.

DOYLE and LOOBY'S view would necessarily be that cleavage polyembryony originated from simple polyembryony independently in six of the seven families recognized by PILGER (**20**); here in the Podocarpaceae it would have arisen at least twice. However, the relationships between these families would not be affected by either theory. So far as embryogeny is concerned, these relationships are indicated by the number of free nuclei in the proembryo before walls appear (whether 32, 16, 8, 4, or 2), by the organization of the early embryonic cells, and by the presence or absence of a prosuspensor or primary suspensor, etc. It must be borne in mind, therefore, that a difference of opinion concerning the status of cleavage polyembryony would not affect the relationships between the major groups of this order, but rather the question as to the relationships of genera within the families or subfamilies.

According to my view, simple polyembryony is attained in at least two directions leading away from the types shown in the centrally placed embryos of the series shown in figures 51 and 52. Passing toward the left (fig. 51), simple polyembryony is attained with a maximum number of binucleate cells arranged in four tiers; passing toward the right (fig. 52), it is attained through reduction in the number of binucleate cells which become arranged in a single tier and finally results in a single binucleate cell.

If one were to choose among the embryogenies represented in the series of early embryos shown in figures 51 and 52 a form nearest the prototype from which the Podocarpaceae have had their origin, neither of the extremes would be selected. The prototype was probably most nearly like one of the intermediate forms, such as *P. usambarensis* and *P. gracilior*. A prototype should have thirty-two free nuclei in the proembryo before walls are formed and nine or more binucleate em-

bryonic cells arranged as in these species of *Podocarpus*. The arrangement found in *Saxegothaea*, *Phyllocladus*, and *P. spicatus* would result from the development of the extremely long and narrow archegonia found here, with a reduction of free nuclei to sixteen, while the types found in the direction of *P. totarra* would result from a similar reduction in the number of free nuclear divisions, also affecting the number of embryonic initials. The number of cells in the prosuspensor appears to have been reduced in both directions. The series of embryonic types shown in figures 51 and 52 may serve as a guide to the true relationships within this family.

Taxonomists who may contemplate a monographic treatment of this family should search for external criteria, correlated with the series shown by embryogeny, as a guide to an improved scheme of phylogeny.

Summary and conclusions

1. This investigation is concerned chiefly with details of early stages in the embryogeny of *Podocarpus dacrydioides* and with a more complete series of stages of *P. urbanii*, *P. macrophyllus maki*, *P. totarra*, and *Phyllocladus alpina*. About a dozen additional species of *Podocarpus* were described from observations of one or a few critical stages each. Even though fragmentary, these observations represent critical stages closely comparable with known stages of the species studied more intensively.

2. The embryos of several species belonging to groups not otherwise available for study were obtained, with satisfactory results, from dried herbarium material. In some of these the successive stages could be recognized by comparison with closely related species having the same general type of embryogeny.

3. A summary of comparable stages in the early embryos of the Podocarpaceae was prepared by drawing upon previous investigations. This summary embraces information concerning the embryogeny of about twenty-two species of the Podocarpaceae belonging to four sections or subgenera of *Podocarpus* and to three other genera. It is highly probable that the extremes in the range of variation within this family are represented in this survey.

4. The genus *Podocarpus* is exceptional among conifers in that it includes several more or less distinct types of embryogeny, whereas other genera usually follow a uniform program.

5. Without exception, all species of the Podocarpaceae thus far examined have binucleate embryonic cells. The number of these ranges from one to about twelve. When there are 9–12 binucleate cells in the proembryo, these may be in a single tier, in two tiers, or in three or four tiers; when the number of binucleate cells is five or less they are usually in a single tier. Apparently the binucleate cells are so organized in the proembryo; they do not increase in number after the prosuspensor begins to elongate.

6. The pair of nuclei in an embryonic cell divide simultaneously or nearly so, giving rise to a condition with four telophasic nuclei (sometimes in tetrahedral position), after which walls appear between all nuclei. Thus the binucleate stage passes directly into a 4-celled condition.

7. An apical cell stage may exist for a period of development in some species of *Podocarpus*. In *Phyllocladus*, however, this stage is very brief or may not appear at all; but after the first appearance of walls in the binucleate cells, an appearance of an apical cell is usually simulated.

8. All the Podocarpaceae thus far examined have prosuspensors. Apparently no primary suspensors are formed, but the embryos that are sometimes cut off from the ends of isolated prosuspensor cells may have the appearance of embryos borne on primary suspensors. However, the latter do not pass through a binucleate stage.

9. The number of cells in the prosuspensor varies from 3–4 in *Saxegothaea* (**10**) and 4–6 in *Phyllocládus* to about eighteen, twenty, or more in *Podocarpus usambarensis* (**5**), *P. gracilior*, and the Nageia group. In the section Protopodocarpus the number of such cells varies from 12–14 in *P. macrophyllus maki* and *P. urbanii* to 7–8 in *P. totarra*.

10. The species having twenty or more cells in the prosuspensor, bearing 7–12 binucleate embryo initials, essentially in a single tier, have indeterminate cleavage polyembryony. The species with 7–11 cells in the prosuspensor, bearing 3–7 binucleate embryo initials in one or two tiers, and those with 12–15 cells in the prosuspensor and 2–3 binucleate embryo initials, usually have determinate cleavage polyembryony. The species having about nine or less cells in the prosuspensor, bearing a single binucleate embryonic cell, or those bearing 7–12 binucleate cells in three or more tiers, usually have simple polyembryony.

11. In the later stages of the embryo systems of species with massive prosuspensors, some of the cells were found to have formed embryonic tissue consisting of internal rows of cells. Rosette cells were observed as isolated cells, sometimes giving rise to small rosette embryos.

12. The behavior of some isolated prosuspensor cells and of some rosette cells, when these are found, suggests that not only the binucleate embryonic cells but all cells of the proembryo are potential embryo initials. In the elongating cells of the prosuspensor the embryo-forming capacity is suppressed.

13. The number of free nuclei, before walls appear in the proembryo, is reflected in the number of cells in the proembryo when the prosuspensor begins to elongate. Although the proembryos have actually been observed in detail in only five species of this family, the type of proembryo (for most of them) may be recognized in its general program of development. From these facts and the proembryos which have actually been observed (**8, 10, 22, 26**), it appears that the number of free nuclei before walls are formed is 16–32.

14. In the organization of cells formed in the early embryo of *P. usambarensis*, *P. gracilior*, and the Nageia group of *Podocarpus*, these forms approach closer than any other conifer to the type of cellular organization found in embryos of the Araucariaceae. The podocarps differ, however, in having a smaller number of cells, in the fact that the embryonic cells are binucleate, and in giving rise to embryo systems with cleavage polyembryony.

15. The embryogenies of the Podocarpaceae, especially within the genus *Podocarpus*, hold great promise—when more completely investigated—of providing the basis for improving the natural system of classification in a taxonomic treatment of this group.

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SEASONAL CHANGES IN BOUND WATER CONTENT OF SOME PRAIRIE GRASSES

WARREN C. WHITMAN[1]

(WITH FOUR FIGURES)

Introduction

In a study of certain grassland vegetation types in western North Dakota, it seemed desirable to attempt to use the bound water content of some of the principal grass species of the various types as a measure of the adaptation of the species to their respective habitats. In this semi-arid region the amount of moisture available for use by the plants is in most cases the principal factor governing the occurrence of different vegetation types. Consequently the measurement of a property which might be an integrated expression of the relation of the plant to its habitat, especially to the available soil moisture, could be expected to throw considerable light on the essential relations between species within the vegetation types and between the types themselves.

This investigation involved weekly determinations of the bound water content of the leaf tissue of seven grass species in four different grassland types during the summer seasons of 1937 and 1938. By bound water is meant that water which remained unfrozen in the leaf tissue after a freezing period of 6 hours at -21.5° C. Various investigators have referred to this water as "unfrozen" (**22**) or as "unfreezable" (**5, 27**).

Review of literature

SPOEHR (**20**) found that when certain species of *Opuntia* were subjected to continued periods of drought the pentosan content of the tissues showed decided increase. It was concluded that the plants were enabled to absorb and retain large quantities of water because of the presence of these hydrophilic colloids of high imbibition capacity. MAXIMOV (**8**) states that in both frost and drought resistance the basis of the resistance lies in the capacity of the protoplasm to withstand the dehydrating influence of a direct or indirect deprivation of water.

NEWTON and MARTIN (**14**) were apparently the first to investigate the relations between drought tolerance, colloidal content, and bound water in plant tissues. They made an extensive study of bound water in relation to drought resistance in crop plants and in native and tame grasses, using the cryoscopic technique of NEWTON and GORTNER (**13**). They found that in southern Alberta the percentage

[1] Mr. ROALD PETERSON and Mr. H. THEO HANSON assisted in the field work of this study.

bound water content of the expressed sap of some of the native grasses correlated directly with the known adaptation of these species to drought conditions. In their studies bound water determinations were made during two seasons, 1925 and 1926.

LEBEDINCEV (7) found that xerophytes growing in a semi-desert region had a higher water-retaining capacity than had mesophytes growing under the same conditions. After repeated wiltings there was a permanent increase in their water-retaining capacity. She concluded, however, that the main role in determining this capacity was played by osmotic substances and not by colloids. In contradiction to this, VASSILIEV and VASSILIEV (25) found that in young wheat plants exposed to drought the hemicelluloses increased and remained high even after a return to normal water supply. The hemicelluloses are of the pentosan group and have marked capacity to swell. BARINOVA (1) also found in the case of sugar beets that an impoverishment of water in the tissues resulted in an increase in hydrophilic colloids in the leaves and roots.

NOVIKOV (15) found that the quantity of bound water in winter wheats, determined when the plants were undergoing drought, was a measure of the drought resistance of the variety. When the plants were growing under optimum moisture conditions, however, they showed no essential differences in water-retaining capacity. He concluded that the increase in this capacity during drought hardening resulted from an increase in water-retaining substances in the plants and not from losses of free water from the plant tissue.

CALVERT (3) studied drought resistance in three varieties of wheat. He determined the total water, percentage free water, percentage bound water, and total solids in the sap expressed from the leaves. The determinations were made at frequent intervals throughout the season, and soil moisture determinations were also made. He found that there was a slight decrease in the percentage of total water in the sap from the beginning to the end of the season, but on the whole the total water remained fairly constant. The percentage of free water in the sap, however, decreased throughout the season. This decline was compensated by a corresponding increase in bound water. This increase was real, whether expressed on the basis of percentage total water or per gram of dry material in the sap. He concluded that the external conditions which reduce the free water per gram of dry material act simultaneously on the plant in such a way as to increase the bound water per gram of dry matter.

In a later study on the sap expressed from the leaves of *Phalaris tuberosa*, CALVERT (2) found that—per gram of dry matter—the total water, the free water, and the bound water were all significantly greater in the morning than in the afternoon. The decrease in free water in the afternoon was considerably greater than the decrease in bound water, and at that time there was actually more bound

water per gram of dry matter than there was free water. These reversed relations apparently resulted from the water deficit created by the increase in transpiration during the day. This work indicates that the bound water content of a species may be directly influenced by the varying conditions of the environment.

In a study of water relations in detached drying leaves of *Bryophyllum calycinum*, WELCH (**27**) found that as the leaf dried the percentage of "unfreezable" or bound water increased, while the percentage of "freezable" or free water decreased. The increase in bound water did not seem to result from an increase in the water-retaining capacity of the leaf tissues, but rather increased merely as the result of loss of free water in the drying process.

SCHOPMEYER (**19**) studied transpiration and physico-chemical properties of leaves of loblolly and shortleaf pine in relation to drought resistance. He found that the more drought-resistant shortleaf pine had only slightly more bound water than loblolly pine when the soil moisture was at the wilting coefficient; and in the other cases when soil moisture was at an optimum value both before and after wilting it had less bound water, even though the total moisture content of this species was greater at all times than that of loblolly pine. He concluded that the greater drought resistance of shortleaf pine could not be attributed to an ability to conserve water either by retarding transpiration or by forming bound water.

Material and methods

Bound water in the leaf tissue was determined by the calorimetric or heat-of-fusion method. The technique followed was essentially similar to that described by GREATHOUSE (**5**). The method was originally used by MÜLLER-THURGAU (**12**) and elaborated by RÜBNER (**17**), and later by THOENES (**24**). THOENES (**24**), ROBINSON (**16**), ST. JOHN (**23**), MEYER (**11**), SAYRE (**18**), STARK (**21**), CHRYSLER (**4**), GREATHOUSE (**5**), and WELCH (**27**) all give excellent discussions of the use of the calorimetric method and the calculation of bound water from calorimetric data.

A sample of leaf tissue weighing approximately 10 gm. was rapidly cut into short lengths and placed in a weighed tinfoil cup. A small lead weight was placed in the cup with the tissue, and the top of the container was folded over and pressed shut. The sample was then weighed and put into a large test tube, which was in turn placed in an acetone-solid carbon dioxide freezing bath, where it was frozen at $-21.5^\circ \pm 0.5^\circ$ C. for 6 hours. Samples were run in duplicate. At the end of about 3 hours the samples were removed from the bath and the containers rapidly slit with a sharp knife. The samples were then returned to the tubes and to the freezing bath. This treatment assured the more rapid establishment of an equilibrium temperature in the calorimeter.

The amount of water frozen in the plant tissues during the freezing period was measured by introducing the sample into a calorimeter containing exactly 250 gm.

of water at slightly above room temperature. The water in the calorimeter was stirred rapidly until an equilibrium temperature was reached. The change in temperature of the water was measured by means of a mercury thermometer graduated to 0.05° F. With the aid of a lens, the reading could be accurately estimated to 0.01° F.

The calorimeter consisted of a pint Dewar flask held in a small wooden case. A space approximately 1 inch wide between the flask and the edges of the case was entirely filled with rock wool, thoroughly insulating the flask. A base of heavy wood supported the case, which fitted closely against the sides and back of the base and was thus held firmly in place. Two vertical steel rods, one on each side of the base, supported the carrying platform, to which motor, stirrer, thermometer, and stopper were attached. The platform was adjusted to move up and down on the steel rods, and it could be locked in any position. This arrangement greatly facilitated the opening and closing of the calorimeter.

A spiral glass stirrer driven by a small electric motor was used to stir the water in the calorimeter. The time required to obtain equilibrium with a 10-gm. sample was about 6–8 minutes.

The water which froze in the samples (free water) was calculated according to the formula given by GREATHOUSE (**5**), with the exception that the use of a lead weight in the samples to insure their complete submergence necessitated the inclusion of an additional factor in the formula. The calorimeter factor, as determined from repeated trials with 10 gm. of water in the tinfoil cup instead of a tissue sample, was 1.094.

The bound water was determined by subtracting the weight of the water frozen from the total water in the sample:

$$W_b = W_W - W_i$$

The total water was secured by subtracting the weight of the dried sample from the green weight. The dry weight and the specific heat of the tissue sample were obtained with the same sample used in the determination of the free water. The samples were dried to constant weight at 88° C. Following the procedure of MEYER (**11**), CHRYSLER (**4**), and others, the specific heat determinations were made on the dried tissue, using benzene in the calorimeter.

The osmotic value of the plant sap was calculated from the depression of the freezing point. The heating method (**10**) was used to kill the tissue. The sap was expressed from the leaf tissue at a pressure of 15,000 pounds per square inch, and the freezing point was determined with the Drucker-Burian microthermometer. The use of this apparatus was originally described by WALTER (**26**). MALLERY (**9**) gives a complete discussion of the method and points out that it is especially

adapted for use in arid regions, since only 1.5–2 ml. of plant sap is necessary in order to make a freezing point determination.

Tissue samples for the bound water and osmotic values were collected in the early morning from the various vegetation types. Each of the four areas was sampled once each week, two being sampled on one day and the other two on the following day. The samples in each type were collected from the area encompassed by a circle with a radius of approximately 50 feet, the point of sampling being at the approximate center of the circle. These samples were also taken once each week.

The tissue samples were placed in pint Mason jars equipped with screw or clamp tops and rubber sealing rings. The jars were then put in a heavy cardboard box and taken back to the laboratory. Samples which could not be handled immediately were placed in the refrigerator. None of the tissue samples was kept for more than 3 hours before preparation, and usually work was begun on them within 15–20 minutes after they reached the laboratory.

Vegetation types

This work was conducted at the Pyramid Park Range Station, located 17 miles southwest of Fryburg, North Dakota, in the broken range country of the Badlands. Four areas, none of which was more than 4 miles from the station, were selected as sample areas. These areas were typical representatives of four of the principal grassland types.

1. UPLANDS TYPE.—One of the areas was on a nearly level uplands terrace 4 miles south of the range station (S.W. $\frac{1}{4}$ sec. 16–138–101). The vegetation was typically that of the grama-needlegrass-sedge type (**6**). The principal species were blue grama grass, *Bouteloua gracilis* H. B. K. Lag.; needle-and-thread, *Stipa comata* Trin. & Rupr.; niggerwool, *Carex filifolia* Nutt.; and western wheatgrass, *Agropyron smithii* Rydb. The species selected for sampling in this area were grama grass, needle-and-thread, and western wheatgrass.

The supply of soil moisture in the uplands type is dependent upon the precipitation received during the season. The subsoil is perpetually dry. In ordinary seasons there is usually no available moisture below 2 feet, and moisture in the upper levels is usually deficient by the middle of July. This is the most xerophytic of the grassland types included in this study.

2. SAGEBRUSH TYPE.—The second sampling area was in the flats along streams and in valleys; it is characterized by the dominance of the gray-colored shrub, *Artemisia cana* Pursh., growing in dense or open stands. This second area was in a typical development of the type found on a low terrace along Davis Creek, about $\frac{1}{2}$ mile from the station (N.E. $\frac{1}{4}$ sec. 11–138–101). The principal grass species are western wheatgrass, grama grass, needle-and-thread, and green needlegrass, *Stipa*

viridula Trin. The same three species were sampled here as in the uplands type—western wheatgrass, grama grass, and needle-and-thread.

The fact that the same species are abundant in the sagebrush type as in the uplands type indicates that there is considerable similarity between the habitats. There are, however, two important differences. The sagebrush type, situated as it is on a terrace flat along a creek bed, has a supply of subsoil moisture, at least for a part of the season. For the most part this moisture is out of reach of the roots of the grasses, but it is tapped by the extensive root system of the sagebrush. In addition, although Davis Creek is dry for most of the season, occasional torrential rains cause the creek to flood over its banks and inundate the terrace on which the sample area was situated. Thus some additional moisture may be received during the season.

3. SANDGRASS TYPE.—The third area selected was on a low sandy ridge about $\frac{1}{4}$ mile west of the uplands type (S.E. $\frac{1}{4}$ sec. 16–138–101). The principal dominant in this type is sandgrass, *Calamovilfa longifolia* (Hook.) Scribn. Important secondary species include the carices *Carex filifolia* Nutt., *C. eleocharis* Wahl., and *C. pennsylvanica* Lam., as well as the grasses, grama grass, needle-and-thread, and Junegrass, *Koeleria cristata* (L.) Pers. The species sampled in this type were sandgrass and Junegrass.

The soil of the sandgrass type is sandy, averaging about 75 per cent sand to a depth of 5 feet. Moisture storage in this type is rather deep because of the coarse nature of the soil material. During most of the season there is an appreciable amount of moisture in the third foot, frequently more than in the upper 2 feet, and presumably the moisture supply is even greater below 3 feet. Unfortunately, records of soil moisture are available only to a depth of 2 feet for the seasons during which the determinations were made.

4. BIG BLUESTEM TYPE.—This type is developed on the lower parts of steep slopes, such as occur in the narrow valleys and draws of the region. It represents the most mesophytic grassland in the region and is really an extension of the eastern tall grass prairie into semi-arid western North Dakota. Vegetation and soil show a high degree of development, which apparently is dependent on receiving more moisture than that furnished by direct precipitation. The additional moisture is received from seepage, runoff, and melting snow drifts. The area selected for sampling was located about 3 miles northwest of the station, on the steep east-facing slope of a narrow valley (N.E. $\frac{1}{4}$ sec. 3–138–101). Big bluestem, *Andropogon furcatus* Muhl.; porcupine grass, *Stipa spartea* Trin.; and side-oats grama, *Bouteloua curtipendula* (Michx.) Torr., are the principal species occurring in this type. Big bluestem and porcupine grass were the species considered in this study.

Experimental results

1. UPLANDS TYPE.—Tables 1 and 2 and figure 1 present the data for the 1937 and 1938 seasons in the uplands type. The outstanding features of the behavior of the species in this type are the marked increases in bound water as percentage of

TABLE 1

MOISTURE IN TISSUE, OSMOTIC VALUE, AND BOUND AND FREE WATER IN SPECIES IN UPLANDS TYPE; 1937 SEASON

DATE	SOIL MOISTURE* (%)	MOISTURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H_2O% TOTAL H_2O	BOUND H_2O GRAMS/100 GM. DRY MATTER	FREE H_2O% TOTAL H_2O	FREE H_2O GRAMS/100 GM. DRY MATTER
		AGROPYRON SMITHII					
6/28	.....	63.0	16.91	20.90	35.56	79.10	134.55
7/3	11.1	62.1	15.60	27.86	45.70	72.14	118.33
7/12	11.8	56.8	17.76	31.09	40.96	68.91	90.79
7/19	9.2	52.1	19.37	35.54	38.64	64.46	70.08
7/26	9.6	45.7	22.97	46.76	39.40	53.24	44.86
8/2	.....	.....	.....	.....	.....	.....	.....
		STIPA COMATA					
6/28	.....	55.8	15.44	31.24	39.46	68.76	86.92
7/3	11.1	54.9	14.43	34.29	41.72	65.71	79.91
7/12	11.8	50.5	15.48	36.16	36.84	63.84	65.04
7/19	9.2	46.7	18.20	37.93	33.22	62.07	54.37
7/26	9.6	34.3	31.00	62.18	32.45	37.82	19.74
8/2	7.8	28.4	33.48	80.90	32.12	19.10	7.58
		BOUTELOUA GRACILIS					
6/28	.....	64.4	13.55	29.71	53.63	70.29	126.90
7/3	11.1	61.8	13.65	29.43	47.57	70.57	114.08
7/12	11.8	59.6	13.82	34.38	50.72	65.62	96.81
7/19	9.2	58.8	16.05	35.27	50.26	64.73	92.23
7/26	9.6	43.5	26.44	66.26	50.93	33.74	25.94
8/2	7.8	26.9	19.56	98.34	36.21	1.66	0.61

* Average wilting coefficient to depth of 3 feet, 12.5%.

total water and the marked decreases in free water percentage, in grams of free water per 100 gm. of dry matter, and in percentage of moisture in the tissue of the different species. Osmotic values show considerable increase. The values of grams of bound water per 100 gm. of dry matter apparently show no general constant tendencies, although the 1938 results indicate a seasonal increase in this value for two of the species.

As shown in figure 1, the decrease in soil moisture during the course of the season was approximately paralleled by a decrease in the percentage of moisture in the tissue. These decreases in total water in the tissue are accompanied by decreases in the percentage free water and in the grams of free water per 100 gm. of dry

TABLE 2

MOISTURE IN TISSUE, OSMOTIC VALUE, AND BOUND AND FREE WATER IN SPECIES IN UPLANDS TYPE; 1938 SEASON

DATE	SOIL MOISTURE* (%)	MOISTURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H_2O % TOTAL H_2O	BOUND H_2O GRAMS/100 GM. DRY MATTER	FREE H_2O % TOTAL H_2O	FREE H_2O GRAMS/100 GM. DRY MATTER
		AGROPYRON SMITHII					
6/28	11.8	62.5	13.40	21.41	35.70	78.59	131.06
7/2	15.7	59.8	12.74	26.12	38.89	73.88	109.81
7/8	13.6	57.3	13.56	24.48	32.91	75.52	101.47
7/17	9.7	51.2	16.28	32.55	35.59	67.47	73.80
7/25	8.5	53.2	17.57	33.17	37.70	66.83	75.89
8/2	7.0	40.7	22.41	48.34	33.23	51.66	35.50
8/6	6.7	41.8	20.52	41.89	30.11	58.11	41.70
		STIPA COMATA					
6/26	11.8	58.4	12.64	16.23	22.74	83.77	117.50
7/2	15.7	55.1	11.69	21.89	26.94	78.11	95.99
7/8	13.6	52.2	12.65	28.29	30.94	71.71	78.57
7/17	9.7	42.8	16.86	37.77	28.33	62.23	46.68
7/25	8.5	42.7	22.10	47.21	35.18	52.79	39.32
8/2	7.0	30.9	29.05	66.34	29.50	33.66	10.34
8/6	6.7	35.9	31.08	50.19	28.15	49.81	27.93
		BOUTELOUA GRACILIS					
6/26	11.8	65.2	8.34	15.51	29.10	84.49	158.50
7/2	15.7	63.8	8.14	19.05	33.60	80.95	142.74
7/8	13.6	60.9	8.29	25.71	40.03	74.29	115.64
7/17	9.7	50.4	13.07	39.57	40.09	60.43	61.82
7/25	8.5	49.3	19.32	47.39	45.93	52.61	51.21
8/2	7.0	36.0	24.11	74.03	41.37	25.97	15.07
8/6	6.7	32.8	27.16	92.92	45.41	7.08	3.14

* Average wilting coefficient to depth of 3 feet, 12.5%.

matter. The grams of bound water per 100 gm. of dry matter do not show any drastic increases or decreases. At the same time values for percentage of bound water increase remarkably.

The outstanding behavior among the individual species is shown by grama

grass. In 1937 the bound water as percentage of total water reached the value of 98.3 by August 2. At this time there was approximately 37 gm. of water per 100

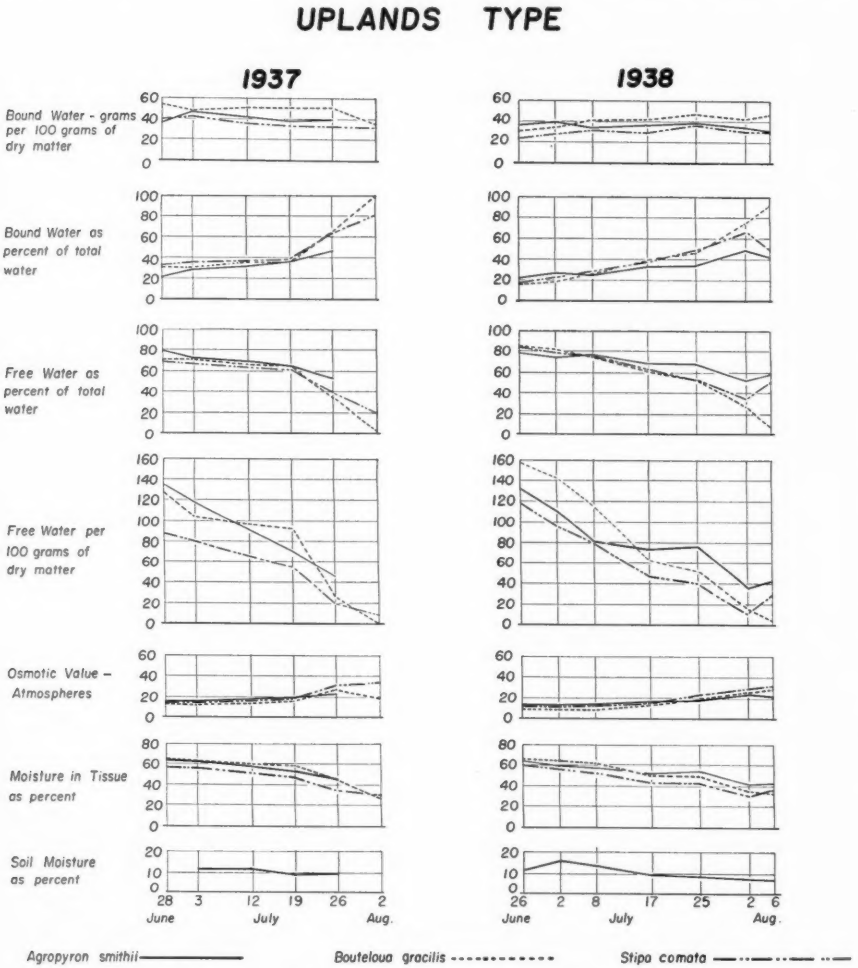
UPLANDS TYPE
1937
1938
Bound Water - grams per 100 grams of dry matter
Bound Water as percent of total water
Free Water as percent of total water
Free Water per 100 grams of dry matter
Osmotic Value – Atmospheres
Moisture in Tissue as percent
Soil Moisture as percent
28 3 12 19 26 2
June July Aug.
26 2 8 17 25 2 6
June July Aug.
Agropyron smithii
Bouteloua gracilis
Stipa comata

FIG. 1.—Changes in bound and free water, osmotic value, and total moisture in leaf tissue of western wheatgrass, blue grama grass, and needle-and-thread in uplands type during summer seasons of 1937 and 1938. Soil moisture also given.

gm. of dry matter in the leaf tissue. In 1938 this same species contained 92.9 per cent bound water on August 6, and the total water content was 48.5 gm. per 100 gm. of dry matter. None of the other species in any of the types equaled these

TABLE 3

SEASONAL AVERAGES OF OSMOTIC VALUE AND BOUND AND FREE WATER FOR ALL SPECIES AND TYPES

Type	Species	1937 season					1938 season				
		Osmotic value (atmos.)	Bound H_2O % total H_2O	Bound H_2O gm./100 gm. dry matter	Free H_2O % total H_2O	Free H_2O gm./100 gm. dry matter	Osmotic value (atmos.)	Bound H_2O % total H_2O	Bound H_2O gm./100 gm. dry matter	Free H_2O % total H_2O	Free H_2O gm./100 gm. dry matter
Uplands	Agropyron smithii	18.52	13.47	40.05	67.57	91.72	16.64	32.56	34.88	67.44	81.32
	Bouteloua gracilis	17.18	48.90	48.22	51.10	76.09	15.49	44.82	39.36	55.12	78.30
	Stipa comata	21.34	47.12	35.97	52.88	52.26	19.44	38.27	28.83	61.73	59.48
	All species	19.01	42.83	41.41	57.18	73.36	17.19	38.55	34.36	61.43	73.03
Sagebrush	Agropyron smithii	25.30	28.88	36.33	71.12	101.20	19.57	29.73	34.22	70.27	97.48
	Bouteloua gracilis	24.50	42.89	49.95	57.11	69.80	17.00	50.58	41.92	49.56	61.10
	Stipa comata	25.05	37.85	38.54	62.15	68.11	27.63	39.97	31.71	60.03	59.22
	All species	24.95	36.54	41.61	63.46	79.70	21.40	40.09	35.95	59.95	72.60
Sandgrass	Calamovilfa longifolia	11.76	32.02	41.79	67.98	88.55	10.73	27.16	36.69	72.84	100.78
	Koeleria cristata	15.84	35.23	43.65	64.77	88.91	15.67	31.60	36.73	64.80	86.48
	All species	13.80	33.62	42.72	66.37	88.73	13.20	29.38	36.71	70.62	93.63
Big bluestem	Andropogon furcatus	9.11	25.69	51.76	74.30	145.30	9.42	22.38	48.94	77.62	170.95
	Stipa spartea	13.13	27.55	32.74	72.45	86.80	14.36	24.27	29.97	75.73	94.10
	All species	11.12	26.62	42.25	73.37	116.05	11.89	23.32	39.45	76.67	132.52

values. In both seasons the bound water per 100 gm. of dry matter was higher in this than in any of the other species in this type. This is shown in figure 1 and in table 3, where the seasonal averages for all species and types are given.

Needle-and-thread was second highest in both seasons in bound water as percentage of total water, while western wheatgrass was the lowest of the three species in this value. In contrast to this, needle-and-thread was lower in grams of bound water per 100 gm. of dry matter at nearly all dates of sampling in both seasons than was western wheatgrass. Needle-and-thread was also lower in grams of free water per 100 gm. of dry matter throughout most of the season. On the basis of the averages for the grams of free water (table 3), western wheatgrass ranked highest and grama grass second. These two species were also higher in total water content than was needle-and-thread.

Osmotic values were fairly uniform in all three species during the seasons. Needle-and-thread, with the lowest total water content and the lowest seasonal values for grams of free water per 100 gm. of dry matter, reached the highest osmotic values at the end of the season. This would indicate that the osmotic effects were not the controlling factor in determining bound water content, since western wheatgrass and grama grass, both with lower average osmotic values than needle-and-thread, were higher in bound water expressed either as percentage or on the dry-weight basis.

In the uplands type during the two seasons the effects of decreasing soil moisture became evident almost from the beginning of the sampling period. By shortly after mid-July the moisture situation became acute, and in both years the species became too dry to sample by the end of the first week in August. Bound water and osmotic values showed their greatest increases after the soil moisture dropped below an approximate 10 per cent level.

2. SAGEBRUSH TYPE.—The data presented in tables 4 and 5 and in figure 2 indicate that there is considerable similarity in behavior of the species sampled in the sagebrush and in the uplands type. The more uniform results obtained for the species in the sagebrush type in 1938 show a greater similarity to the results obtained for the same species in the uplands type than do the 1937 results. In general, the same marked increases in bound water as percentage of total water and the marked decreases in percentage free water, grams of free water per 100 gm. of dry matter, and percentage of moisture in the tissue were shown by these species. With the exception of the behavior of grama grass in 1937, the values of grams of bound water per 100 gm. of dry matter were fairly uniform in the species during both seasons.

Grama grass again had the highest values for bound water as percentage of total water, and in 1938 a maximum of 92.1 per cent for this value was observed on August 2. In table 3 it is shown that the averages for percentage bound water were

higher in this species during both seasons than in any of the other types. As in the uplands type, needle-and-thread was second highest in percentage bound water, while western wheatgrass again had lower average values for this factor than either of the other species.

Grams of bound water per 100 gm. of dry matter was higher in grama grass than in the other species, but in the 1937 season needle-and-thread averaged below western wheatgrass in regard to this value, although averaging higher in the 1938 season. In the uplands type, values for grams of bound water averaged higher during both seasons in needle-and-thread than they did in western wheatgrass.

Figure 2 and tables 4 and 5 show that in the sagebrush type, grama grass was lower in percentage of free water and in grams of free water per 100 gm. of dry matter throughout most of the sampling period than were needle-and-thread and western wheatgrass. Needle-and-thread was intermediate between grama grass and western wheatgrass in free water per 100 gm. of dry matter for most of the season. The free water in all species in both uplands and sagebrush types decreased at a rather rapid rate from the beginning of the sampling period to its end.

The osmotic values of all species in the type showed a general increasing trend during the season. The average osmotic values in the species in the sagebrush type were higher than the average values of the species in the uplands type. The total moisture in the tissue decreased in all species in both types, the rate and extent of decrease being similar during comparable seasons.

Average soil moisture to a depth of 2 feet in the sagebrush type was not once above the calculated wilting coefficient of 9.2 per cent during the sampling period in 1937. In the 1938 season the average soil moisture was above the calculated wilting coefficient until about the middle of July, and in this season the marked decreases in percentage of moisture in the tissue and in percentage of free water begin at about this time.

3. SANDGRASS TYPE.—The species sampled in this type showed a behavior different from those sampled in the uplands and sagebrush types. The marked increases in percentage bound water with the progress of the season were not observed in Junegrass or in sandgrass. Junegrass showed relatively greater changes in percentage bound water, osmotic value, total moisture, and in percentage and grams of free water than did sandgrass. Yet in general the range of the values was considerably smaller than the range of these same values in the species in the sagebrush and upland types (tables 6, 7; fig. 3). They appear to be primarily related to moisture conditions and to the type of root systems of the species sampled in the sandgrass type. The sandy soil of the sandgrass area permits relatively deep moisture storage, and the wilting coefficient is lower, being only 7.1 per cent. The moisture in the upper 2 feet of the soil was below this value from the middle

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of July to the end of the sampling period during 1937 and in 1938 was not above the wilting coefficient after the end of July.

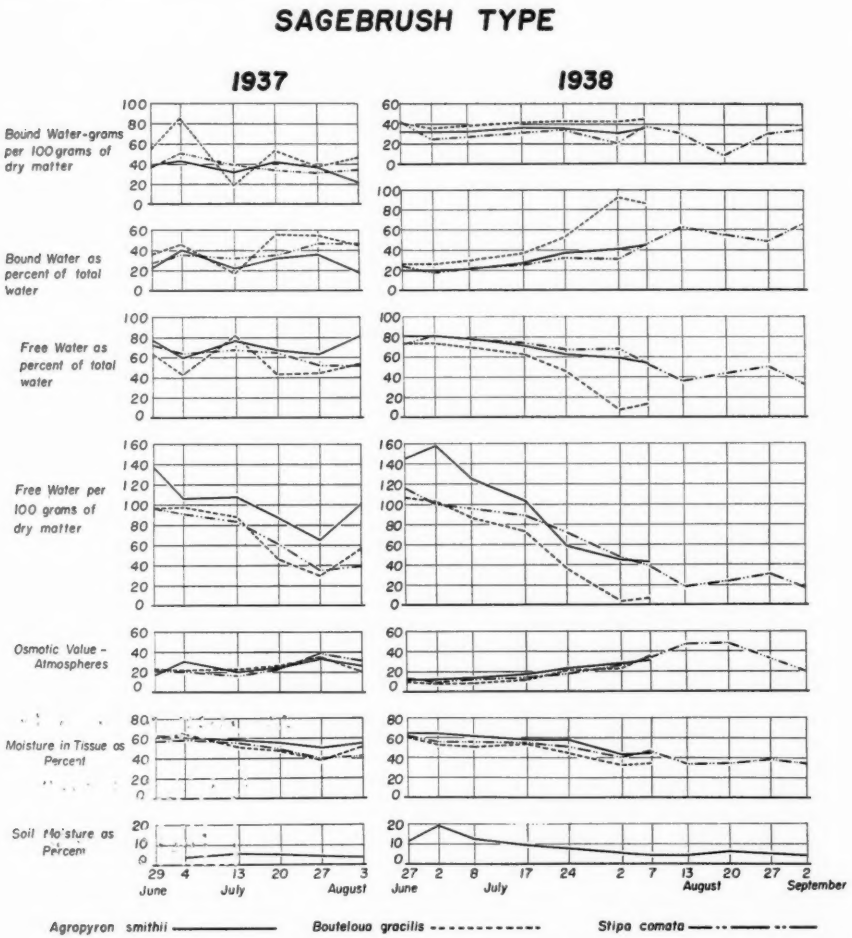
SAGEBRUSH TYPE
1937
1938
Bound Water-grams per 100 grams of dry matter
Bound Water as percent of total water
Free Water as percent of total water
Free Water per 100 grams of dry matter
Osmotic Value - Atmospheres
Moisture in Tissue as Percent
Soil Moisture as Percent
29 4 13 20 27 3
June July August
27 2 8 17 24 2 7 13 20 27 2
June July August September
Agropyron smithii
Bouteloua gracilis
Stipa comata

FIG. 2.—Changes in bound and free water, osmotic value, and total moisture in leaf tissues of western wheatgrass, blue grama grass, and needle-and-thread in sagebrush type during summer seasons of 1937 and 1938. Soil moisture also included.

Junegrass, which has a relatively shallow root system, was apparently unable to utilize the deeper moisture, and when moisture in the upper soil levels was largely depleted this species became too dry to sample. The values for percentage bound water, free water, osmotic concentration, and total moisture in the tissue

seem to reflect to considerable extent the soil moisture situation. In the case of sandgrass there was only a slight decrease in the total moisture in the tissue during the sampling period. Increases in the percentage of bound water were small or nonexistent in this species. Values for grams of bound water per 100 gm. of dry

TABLE 4

MOISTURE IN TISSUE, OSMOTIC VALUE, AND BOUND AND FREE WATER IN SPECIES IN SAGEBRUSH TYPE; 1937 SEASON

Date	Soil moisture* (%)	Moisture in tissue (%)	Osmotic value (atmos.)	Bound H_2O % total H_2O	Bound H_2O grams/100 gm. dry matter	Free H_2O % total H_2O	Free H_2O grams/100 gm. dry matter
		Agropyron smithii					
6/29	.....	63.6	17.60	22.34	39.58	77.66	137.58
7/4	4.0	60.1	31.20	40.92	43.90	59.08	106.45
7/13	5.3	58.4	20.15	22.88	32.14	77.12	108.34
7/20	5.1	56.6	23.74	32.56	42.40	67.44	87.83
7/27	4.1	50.7	32.71	36.44	37.42	63.56	65.27
8/3	3.6	55.4	26.38	18.15	22.56	81.85	101.71
		Stipa comata					
6/29	.....	57.4	20.44	27.89	37.56	72.11	97.13
7/4	4.0	58.9	20.10	36.03	51.68	63.97	91.76
7/13	5.3	55.1	16.18	32.29	39.66	67.71	83.17
7/20	5.1	48.9	23.52	35.76	34.28	64.24	61.57
7/27	4.1	40.4	38.37	47.36	32.08	52.64	35.65
8/3	3.6	43.0	31.71	47.75	35.98	52.25	39.37
		Bouteloua gracilis					
6/29	.....	60.5	22.34	36.14	55.36	63.86	97.83
7/4	4.0	64.9	20.21	46.77	86.51	53.23	98.45
7/13	5.3	51.8	21.74	17.93	19.26	82.07	88.14
7/20	5.1	48.8	25.95	56.32	53.72	43.68	46.28
7/27	4.1	40.6	36.65	55.02	37.68	44.98	30.80
8/3	3.6	51.1	20.10	45.16	47.17	54.84	57.28

* Average wilting coefficient to depth of 3 feet, 9.2%.

matter in sandgrass fluctuated somewhat but seemed to show no constant significant tendencies (fig. 2). The grams of free water per 100 gm. of dry matter in this species showed a consistent decreasing trend with advance of the season.

Table 3 shows that the average value for percentage bound water of the two species in the sandgrass type was lower than the averages for percentage bound

water of the species in the uplands and sagebrush types. The grams of bound water per 100 gm. of dry matter for the two sandgrass species averaged higher,

TABLE 5

MOISTURE IN TISSUE, OSMOTIC VALUE, AND BOUND AND FREE WATER IN SPECIES IN SAGEBRUSH TYPE; 1938 SEASON

DATE	SOIL MOISTURE* (%)	MOISTURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H_2O % TOTAL H_2O	BOUND H_2O GRAMS/100 GM. DRY MATTER	FREE H_2O % TOTAL H_2O	FREE H_2O GRAMS/100 GM. DRY MATTER
		AGROPYRON SMITHII					
6/27	11.8	64.0	11.36	18.31	32.65	81.69	145.69
7/2	19.3	64.0	12.41	18.32	32.85	81.68	158.43
7/8	12.9	61.5	13.55	21.23	33.96	78.75	125.99
7/17	9.9	58.6	17.02	26.17	37.12	73.83	104.79
7/24	8.0	48.6	23.57	37.73	35.76	62.27	59.04
8/2	5.9	43.4	27.63	40.98	31.50	59.02	45.31
8/7	4.9	44.0	31.47	45.37	35.69	54.63	43.14
		STIPA COMATA					
6/27	11.8	61.4	13.35	26.64	42.56	73.36	116.12
7/2	19.3	55.7	9.51	19.91	25.06	80.09	100.71
7/8	12.9	55.2	12.84	22.24	27.54	77.76	96.18
7/17	9.9	54.7	14.76	26.06	31.42	73.94	89.41
7/24	8.0	51.8	18.32	32.53	34.90	67.47	72.38
8/2	5.9	40.4	26.69	31.47	21.86	68.53	46.18
8/7	4.9	45.1	34.01	46.12	38.00	53.88	39.10
8/13	4.7	33.3	47.73	63.50	31.82	36.50	18.27
8/20	6.1	34.6	48.57	55.14	29.30	44.86	23.84
8/27	5.4	38.3	33.80	49.81	30.93	50.19	31.18
9/2	4.6	34.8	44.39	66.23	35.43	33.77	18.07
		BOUTELOUA GRACILIS					
6/27	11.8	61.5	10.60	26.13	41.74	73.87	117.91
7/2	19.3	57.9	8.13	26.45	36.47	73.55	101.45
7/8	12.9	55.9	9.01	30.75	39.06	69.25	87.95
7/17	9.9	53.7	12.27	37.14	43.00	62.86	73.19
7/24	8.0	44.2	21.59	53.88	43.61	46.12	36.33
8/2	5.9	32.1	23.03	92.06	43.49	7.94	3.75
8/7	4.9	34.7	34.35	86.65	46.09	13.35	7.10

* Average wilting coefficient to depth of 3 feet, 9.2%.

however, than this same value for the species mentioned in the previous discussion. The average values for grams of free water per 100 gm. of dry matter also were considerably higher in Junegrass and sandgrass than they were in western

TABLE 6

MOISTURE IN TISSUE, OSMOTIC VALUE, AND BOUND AND FREE WATER IN SPECIES IN SANDGRASS TYPE; 1937 SEASON

DATE	SOIL MOISTURE* (%)	CALAMOVILFA LONGIFOLIA						KOELERIA CRISTATA					
		MOISTURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H_2O % TOTAL H_2O	BOUND H_2O GRAMS/100 GM. DRY MATTER	FREE H_2O % TOTAL H_2O	FREE H_2O GRAMS/100 GM. DRY MATTER	MOISTURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H_2O % TOTAL H_2O	BOUND H_2O GRAMS/100 GM. DRY MATTER	FREE H_2O % TOTAL H_2O	FREE H_2O GRAMS/100 GM. DRY MATTER
6/28	.....	61.6	11.80	32.34	51.77	67.66	108.33	65.2	13.89	29.04	54.54	70.96	133.23
7/3	6.5	63.5	8.34	34.60	60.25	65.40	113.88	63.9	12.59	29.91	53.06	70.09	124.35
7/12	7.1	57.2	9.80	27.68	37.08	72.31	96.87	59.2	13.54	23.87	34.69	76.13	110.66
7/19	5.2	55.8	11.14	38.27	48.52	61.73	78.25	54.1	15.54	35.13	41.41	64.87	76.46
7/26	4.7	55.5	13.60	30.83	38.40	69.17	86.15	46.5	20.45	49.50	42.99	50.50	43.86
8/2	4.1	55.4	11.74	25.10	31.22	74.90	93.17	44.5	19.04	43.95	35.21	56.05	44.91
8/9	3.7	52.5	14.25	31.44	34.80	68.56	75.89	.....	42.11	.....	.....	.....	.....
8/16	3.2	55.0	11.96	29.82	36.50	70.18	85.91	.....	.....	.....	.....	.....	.....
8/23	3.6	52.5	12.80	36.63	40.46	63.37	70.00	.....	.....	.....	.....	.....	.....
8/30	3.7	53.7	12.21	33.49	38.78	66.51	77.02	.....	.....	.....	.....	.....	.....

* Average wilting coefficient to depth of 3 feet, 7.1%

TABLE 7

MOISTURE IN TISSUE, OSMOTIC VALUE, AND BOUND AND FREE WATER IN SPECIES IN SANDGRASS TYPE; 1938 SEASON

DATE	SOIL MOISTURE* (%)	CALAMOVILFA LONGIFOLIA						KOELERIA CRISTATA					
		MOISTURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H_2O % TOTAL H_2O	BOUND H_2O GRAMS/100 GM. DRY MATTER	FREE H_2O % TOTAL H_2O	FREE H_2O GRAMS/100 GM. DRY MATTER	MOISTURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H_2O % TOTAL H_2O	BOUND H_2O GRAMS/100 GM. DRY MATTER	FREE H_2O % TOTAL H_2O	FREE H_2O GRAMS/100 GM. DRY MATTER
6/26	11.7	61.6	8.49	20.51	33.10	79.49	127.41	63.9	10.73	25.03	44.36	74.97	132.81
7/2	13.6	60.8	7.78	20.71	32.22	79.29	123.25	63.2	10.17	18.61	32.01	81.39	139.88
7/8	11.0	60.4	8.27	20.84	32.39	79.16	123.06	61.7	10.29	22.35	36.01	77.65	125.11
7/17	7.6	59.0	6.90	29.14	41.96	70.86	102.04	55.1	13.79	26.95	33.19	73.05	89.86
7/25	6.6	59.7	7.93	26.70	39.68	73.30	108.93	59.8	10.82	31.37	46.75	68.63	102.21
8/2	5.2	55.1	13.62	25.17	30.88	74.83	91.69	51.0	21.90	35.38	36.88	64.62	67.23
8/6	5.0	56.7	9.76	32.19	42.20	67.81	88.77	52.0	12.83	34.16	37.21	65.84	71.80
8/13	4.7	53.5	12.16	30.71	35.46	69.29	79.96	38.4	21.80	50.08	31.38	49.92	1.38
8/20	4.6	55.3	21.41	27.35	33.83	72.65	90.28	44.6	28.72	40.44	32.65	59.56	48.09
8/27	4.4	54.3	10.42	33.05	40.64	66.95	82.29	.....	.....	.....	.....	.....	.....
9/2	4.4	55.9	11.31	32.40	41.20	67.60	90.94	.....	.....	.....	.....	.....	.....

* Average wilting coefficient to depth of 3 feet, 7.1%.

wheatgrass, grama grass, and needle-and-thread in the uplands and sagebrush types. Thus there would seem to be a relation between high values for free water, high values for grams of bound water, and high values for total moisture content

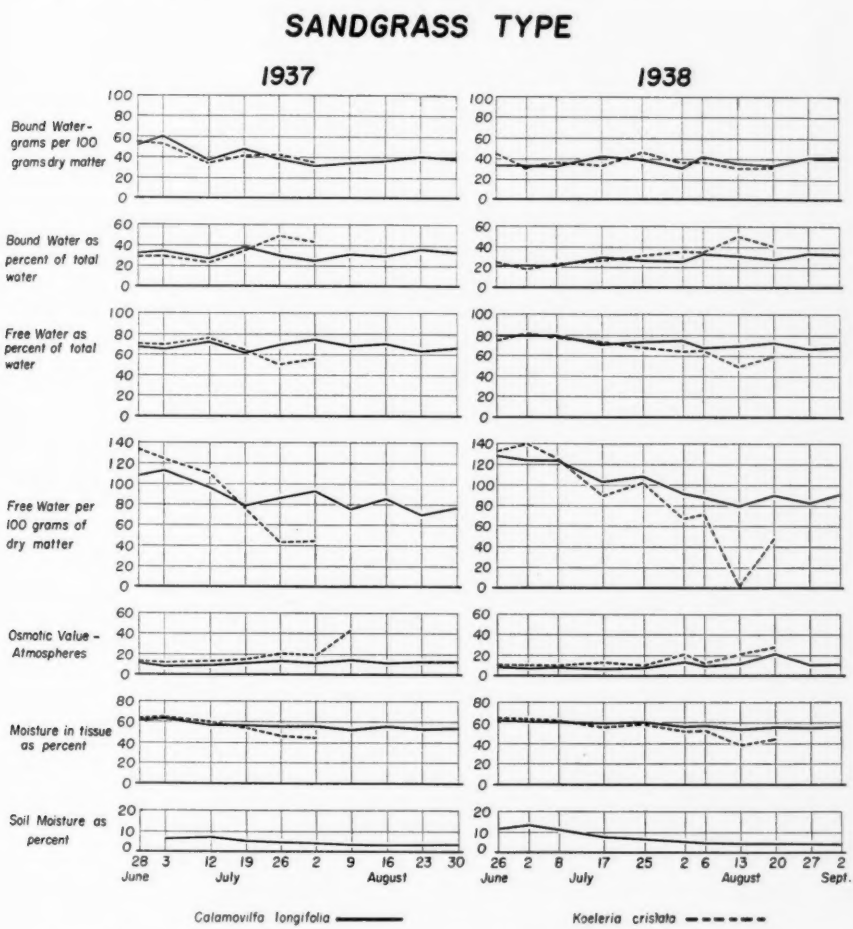
SANDGRASS TYPE
1937
1938
Bound Water - grams per 100 grams dry matter
Bound Water as percent of total water
Free Water as percent of total water
Free Water per 100 grams of dry matter
Osmotic Value - Atmospheres
Moisture in tissue as percent
Soil Moisture as percent
28 3 12 19 26 2 9 16 23 30
June July August
26 2 8 17 25 2 6 13 20 27 2
June July August Sept.
Calamovilfa longifolia
Koeleria cristata

FIG. 3.—Changes in bound and free water, osmotic value, and total moisture in leaf tissues of sandgrass and Junegrass in sandgrass type during summer seasons of 1937 and 1938. Soil moisture also given.

of the tissue. On the other hand, high values for percentage bound water seem to be related to relatively low values for free water and total moisture content of the tissue.

4. BIG BLUESTEM TYPE.—The behavior of the species in this type, as illustrated in figure 4, is typically that to be expected when no stress is placed upon the plants by scarcity of available soil moisture. Although the calculated wilting coefficient

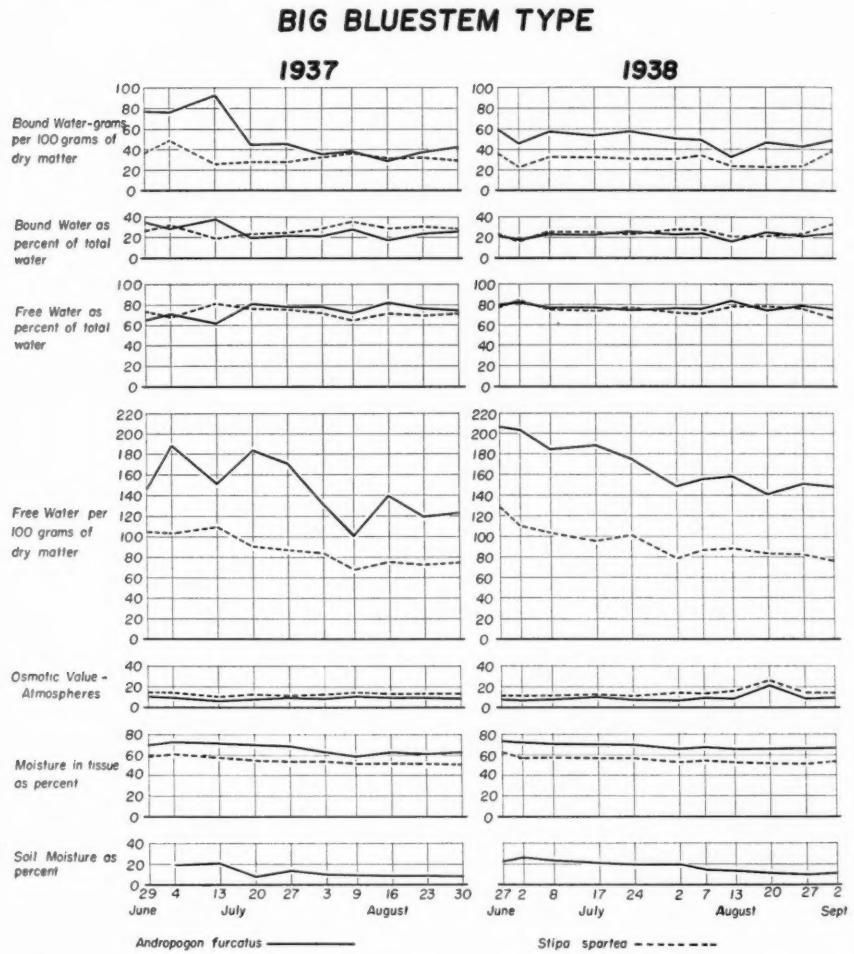
BIG BLUESTEM TYPE
1937
1938
Bound Water-grams per 100 grams of dry matter
Bound Water as percent of total water
Free Water as percent of total water
Free Water per 100 grams of dry matter
Osmotic Value - Atmospheres
Moisture in tissue as percent
Soil Moisture as percent
29 4 13 20 27 3 9 16 23 30
June July August
27 2 8 17 24 2 7 13 20 27 2
June July August Sept
Andropogon furcatus
Stipa spartea

FIG. 4.—Changes in bound and free water, osmotic value, and total moisture in leaf tissues of big bluestem and porcupine grass in big bluestem type during summer seasons of 1937 and 1938. Soil moisture also included.

for the soil in this area is 10 per cent, the soil moisture remained above this point until the first part of August in 1937, and in 1938 it did not fall below the level of the wilting coefficient during the entire sampling period.

TABLE 8

MOISTURE IN TISSUE, OSMOTIC VALUE, AND BOUND AND FREE WATER IN SPECIES IN BIG BLUESTEM TYPE; 1937 SEASON

DATE	SOIL MOISTURE* (%)	ANDROPOGON FURCATUS						STIPA SPARTEA					
		MOISTURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H_2O % TOTAL H_2O	BOUND H_2O GRAMS/100 GM. DRY MATTER	FREE H_2O % TOTAL H_2O	FREE H_2O GRAMS/100 GM. DRY MATTER	MOISTURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H_2O % TOTAL H_2O	BOUND H_2O GRAMS/100 GM. DRY MATTER	FREE H_2O % TOTAL H_2O	FREE H_2O GRAMS/100 GM. DRY MATTER
6/29	.....	69.0	10.30	34.62	77.12	65.38	145.54	58.6	14.81	26.07	36.97	73.93	104.82
7/4	19.3	72.6	9.38	28.75	76.10	71.25	188.57	60.3	14.16	31.95	48.55	68.05	103.39
7/13	20.3	71.0	6.16	37.94	92.79	62.06	151.75	57.4	10.85	18.74	25.23	81.26	109.39
7/20	8.5	69.5	7.40	19.64	44.75	80.36	183.06	54.2	12.45	23.39	27.67	76.61	90.61
7/27	14.2	68.3	9.49	21.15	45.67	78.85	170.22	53.5	11.69	24.32	27.96	75.68	86.99
8/3	10.6	62.5	8.02	21.14	35.26	78.86	131.56	53.7	12.04	28.25	32.82	71.75	83.34
8/9	9.3	58.2	10.77	27.46	38.26	72.54	101.06	51.1	14.50	35.32	36.98	64.68	67.71
8/16	8.5	62.8	9.73	17.41	29.34	82.59	139.19	51.3	13.38	28.58	30.07	71.42	75.15
8/23	8.8	60.9	9.95	23.42	36.48	76.58	119.31	51.1	13.70	30.66	31.98	69.34	72.33
8/30	8.3	62.2	8.96	25.42	41.84	74.58	122.79	50.8	13.72	28.21	29.16	71.79	74.23

* Average wilting coefficient to depth of 36 inches, 10.0%.

TABLE 9

MOISTURE IN TISSUE, OSMOTIC VALUE, AND BOUND AND FREE WATER IN SPECIES IN BIG BLUESTEM TYPE; 1938 SEASON

DATE	SOIL MOISTURE* (%)	ANDROPOGON FURCATUS						STIPA SPARTEA					
		MOISTURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H_2O % TOTAL H_2O	BOUND H_2O GRAMS/100 GM. DRY MATTER	FREE H_2O % TOTAL H_2O	FREE H_2O GRAMS/100 GM. DRY MATTER	MOISTURE IN TISSUE (%)	OSMOTIC VALUE (ATMOS.)	BOUND H_2O % TOTAL H_2O	BOUND H_2O GRAMS/100 GM. DRY MATTER	FREE H_2O % TOTAL H_2O	FREE H_2O GRAMS/100 GM. DRY MATTER
6/27	22.8	73.3	7.76	21.76	59.17	78.24	216.41	62.1	11.16	21.88	35.94	78.12	128.19
7/2	26.5	71.4	6.97	17.43	45.10	82.57	213.68	57.0	11.22	16.98	22.50	83.02	110.20
7/8	23.1	70.8	7.61	23.85	57.37	76.15	184.22	57.6	11.81	24.21	32.90	75.79	103.06
7/17	20.3	70.7	9.77	22.21	53.41	77.79	188.29	56.2	12.50	25.42	32.72	74.58	95.95
7/24	19.7	70.0	7.07	24.65	57.47	75.35	175.85	56.9	11.40	23.46	30.95	76.54	101.04
8/2	19.1	65.8	6.57	23.35	45.25	76.65	148.48	52.9	14.48	28.00	30.82	72.00	78.98
8/7	14.4	67.1	9.09	24.18	49.55	75.82	155.17	54.8	13.96	28.68	34.84	71.32	86.74
8/13	13.3	65.6	8.83	16.81	32.07	83.19	158.33	52.9	16.20	21.13	23.82	78.87	88.40
8/20	11.4	65.1	21.63	25.03	46.96	74.97	140.12	51.6	26.19	21.61	23.04	78.39	83.52
8/27	10.0	66.0	8.84	21.93	42.74	78.07	151.72	51.3	14.36	22.30	23.60	77.70	82.04
9/2	11.1	66.4	9.48	24.96	49.31	75.04	148.22	53.5	14.68	33.26	38.50	66.74	76.99

* Average wilting coefficient to depth of 36 inches, 10.0%.

The data do not indicate any appreciable increase or decrease in percentage bound water and percentage free water. The osmotic values during the sampling period in 1937 remained on practically the same level, while in 1938 they showed only slight increases. The moisture percentage in the tissue of both species showed small but consistent decreases, and the decrease in grams of free water per 100 gm. of dry matter is large enough in both species to possess considerable significance. Grams of bound water per 100 gm. of dry matter show a fairly narrow range, with the exception of the values obtained for big bluestem in the early part of the 1937 season.

The entire behavior pattern of these species, as contrasted with the patterns obtained in the uplands and sagebrush types (figs. 1, 2), indicates a slow loss of moisture from the plant tissue with increasing maturity, rather than a relatively abrupt loss of moisture occasioned by the moisture level in the soil being reduced below the wilting coefficient. There is considerable similarity between the behavior of the species in the big bluestem type and in the sandgrass type. Sandgrass especially seemed to behave much as did big bluestem and porcupine grass.

Porcupine grass was considerably lower throughout the season in percentage moisture in the tissue and in grams of free water per 100 gm. of dry matter than was big bluestem (fig. 4). On the other hand, it was higher in osmotic value and averaged higher in percentage bound water for both seasons than did big bluestem (table 3). Big bluestem, with a much higher total moisture level and much greater values for grams of free water per 100 gm. of dry matter, showed higher values for grams of bound water per 100 gm. of dry matter than did porcupine grass. Not only this, but the average values of grams of bound water per 100 gm. of dry matter in big bluestem were higher than for any other species in any of the types, indicating again that the absolute amounts of bound water are closely related to the total moisture content of the tissue.

The average values for the various factors for both species in this type (table 3) show some significant differences when contrasted with the average values for the species in the other types. The average osmotic value for both species in the big bluestem type was considerably lower than the averages in any of the other types. The average for bound water percentage was also lower. On the other hand, the average bound water content of the two species expressed in terms of grams per 100 gm. of dry matter was approximately on the same level in the 1937 season as it was for the species in the other types. In the 1938 season the average of grams of bound water for the species in the big bluestem type was appreciably higher than the averages of this value for the species in the other types. As previously mentioned, the reason for this lies in the unusually high values for this factor shown by big bluestem. Another outstanding difference between the types is shown by the

much higher values for grams of free water recorded for the species in the big bluestem type. This again is the result of the remarkably higher average values of grams of free water in big bluestem.

Discussion

The results of this study seem to indicate that the bound water expressed as percentage of total water in the tissue, the percentage of total water in the tissue, the percentage of free water, and the grams of free water per 100 gm. of dry matter give the best expression of the responses of the plant species to the conditions of their habitats.

In the uplands type, where soil moisture is reduced below the wilting coefficient relatively early and the plants are placed under extreme stress for water, the species showed marked increases in bound water as percentage of total water and marked decreases in percentage of free water and in grams of free water per 100 gm. of dry matter.

In the sagebrush type, where conditions are similar to those in the uplands type, the species also showed marked increases in bound water as percentage of total water and marked decreases in percentage free water, and in grams of free water per 100 gm. of dry matter, as the season progressed. Both types represent relatively xerophytic habitats.

In the sandgrass type, which is not so xerophytic as the uplands and sagebrush types, only slight increases in percentage bound water occurred in the leaf tissue of the species during the course of the sampling period. Sandgrass showed less change than did Junegrass in percentage bound water and in percentage free water. Fairly large decreases in grams of free water per 100 gm. of dry matter were observed in both species, with larger decreases shown by Junegrass than by sandgrass.

In the big bluestem type, which is more mesophytic than any of the other three types, and where soil moisture was adequate throughout the period of sampling, increases in percentage bound water were relatively small, as were decreases in percentage free water and in grams of free water per 100 gm. of dry matter in the leaf tissue of the species.

The bound water expressed as grams per 100 gm. of dry matter did not seem to give an easily interpretable expression of the response of the species to the conditions of the habitat. Rather, this value seemed to be a characteristic of the individual species, which may or may not have been related to its ability to endure xerophytic conditions. In practically all species in all the types, the absolute amount of bound water (that is, bound water expressed on a dry-weight basis) tended to remain on somewhat the same level throughout the sampling period. This was illustrated especially in the species in the uplands and sagebrush types. Despite the large increases in percentage bound water, the values of grams of

bound water per 100 gm. of dry matter in the species in these two types in most cases showed only relatively small increases or decreases during the sampling period. In the sandgrass and big bluestem types the same general behavior was exhibited by the species, except that it was not so striking because the range of change in the other factors was so much smaller.

With the absolute amounts of bound water in the leaf tissue of the different species remaining on somewhat the same level, it is obvious that any losses in free water will be reflected as increases in percentage bound water. Thus the increase in percentage bound water in the species as the season progresses is primarily an accompaniment to the drying of the tissue. The actual amount of water held in the bound state in the plant tissue may even be decreasing under these conditions.

In habitats where soil moisture is inadequate, therefore, the tissues of the plants will be subjected to greater drying influences and the species will have a lower total water content, less free water per gram of dry matter, and consequently, higher percentages of bound water. At the beginning of the sampling periods, when soil moisture was adequate in all types and the percentage of moisture in the tissue was at a relatively high level in all species, values of percentage bound water were relatively low and approximately on the same general level for all species in all four types.

With the progressive seasonal reduction in soil moisture in the types, the leaf tissues of the plants began to lose moisture. These losses in total water in the tissue and in free water are reflected as increases in the bound water as percentage of the total water. The percentage of bound water in a given species is then a reflection of the extent to which drying of the tissue has taken place. Since this in turn is related to soil moisture, there seems to be a somewhat close relationship between soil moisture and percentage bound water. Consequently, the bound water percentage is primarily a reflection of the relative degree of dryness of the habitat, and not a measure of the adaptation of that species to a dry habitat.

This does not obviate the fact that the absolute bound water content of a species may be related to its ability to withstand drought conditions. It does indicate that this relationship is complex and the nature of it may not be revealed by gross measurements of the bound water content of the leaf tissues of a species growing in a given habitat.

Summary

1. A study of the bound and free water content of several prairie grasses in four representative grassland types in western North Dakota was made during the summer seasons of 1937 and 1938. The principal grass species in each of the four types were sampled once each week during the season for determinations of total water content, osmotic value of expressed sap, and relative proportions of bound and free water.

2. The bound water percentage, percentage free water, and grams of free water per 100 gm. of dry matter seem to give the best expression of the responses of the species to the conditions of their habitats.

3. Increases in bound water as percentage of total water resulted from losses in free water caused by progressive drying of the plant tissues and not from increases in the water-retaining capacity of the leaves. The bound water percentage is thus a reflection of the relative degree of dryness of the habitat and not a measure of the inherent adaptation of that species to a dry habitat.

4. The absolute amount of bound water present in the plant tissues, expressed as grams of bound water per 100 gm. of dry matter, did not seem to give an easily interpretable expression of the responses of the species to habitat conditions. This value seemed to be characteristic of the individual species and in each remained on somewhat the same level during the sampling period. There were specific differences in absolute amounts of bound water between species, but these differences apparently were not a direct measure of the ability of the species to tolerate xerophytic conditions.

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FARGO, NORTH DAKOTA

LITERATURE CITED

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CHEMICALLY INDUCED PARTHENOCARPY IN CERTAIN HORTICULTURAL PLANTS, WITH SPECIAL REFERENCE TO THE WATERMELON[1]

C. Y. WONG

(WITH EIGHT FIGURES)

Introduction

Natural parthenocarpy is of common occurrence in banana, citrus, vinifera grape, Chinese persimmon, and English forcing cucumber. Induced or stimulative parthenocarpy in many species and varieties has been observed to occur as a result of certain internal and external influences. Since parthenocarpic fruits are considered desirable, efforts have been made: (*a*) to obtain seedless fruits by breeding or selection, (*b*) to induce parthenocarpic in place of non-parthenocarpic fruits, and (*c*) to increase the percentage of parthenocarpic fruits where some occur normally.

The object of this study was to determine the possibility of inducing parthenocarpy by the use of synthetic growth substances in some horticultural crops, especially the watermelon. More specifically it aimed to determine: (1) the response of certain horticultural plants to parthenocarpic and apomictic phenomena, (2) the kinds and concentrations of growth substances that give best results, (3) the fruit and seed development in parthenocarpic as compared with normal seed-bearing fruit, and (4) the possibility for practical application of the results obtained.

Review of literature

As early as 1902, MASSART (**11**) placed dead pollen upon the stigma of an orchid and observed a slight growth of the ovary. HARTLEY (**10**) obtained parthenocarpic fruit of tobacco by the use of *Azalea* pollen. FITTING (**2, 3**), using dead and living pollen and pollen extracts, was able to cause some slight growth in the ovary of several species of orchids. WINGE (**15**) found that large fruits with sterile seeds could be produced in hops pollinated with hemp or *Urtica* pollen.

In attempts to produce parthenocarpy by cross pollination and other means, only some of which were successful, YASUDA (**16, 17, 18, 20**) obtained parthenocarpic eggplant, tomato, and pepper fruits by pollination with pollen other than their own. He also obtained satisfactory results by injecting aqueous extracts of pollen into the ovary. In cucumber and a solanaceous plant, however, partheno-

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carpic development did not take place in flowers that opened early in the season, although it did appear at a later date.

In cases where parthenocarpic fruit developed, the pollen tubes grew into the style but did not reach the ovary. In later studies (**19**) the flowers of eggplant and cucumber were emasculated and pollinated with their own well-developed pollen grains. The pollinated stigmas and styles were then cut off at different periods following pollination. When the operation was delayed (24 hours in eggplant and 9 hours in cucumber), fertilization occurred and the ovaries developed into normal seeded fruits. If the operation was so timed that the majority of the pollen tubes reached the base of the style, some seedless fruits resulted. It was concluded that the pollen grains or pollen tubes produced some chemical substances which caused parthenocarpic development of the ovaries. Parthenocarpy, phenospermy (stenospermy), and parthenogenesis were believed to be caused by the action of the same stimulating substance. It was further concluded that parthenogenesis would take place when the pollen-tube substance reached the ovules and stimulated them without fertilization. On the other hand, when the substance arrived at the base of the style or at the ovaries and stimulated the ovarian tissue alone, parthenocarpy would occur. Phenospermy may take place in an intermediate situation. By use of pollen extracts, GUSTAFSON (**7**) produced parthenocarpy in eggplant, pepper, cucumber, and certain ornamental plants.

Parthenocarpy induced by growth substances was first accomplished by GUSTAFSON (**6**) in 1936 by treating the pistils with known hormones in lanolin paste. He obtained normal-sized parthenocarpic fruits in tomato, *Petunia*, and *Salpiglossis* when the pistils were treated with indole-3n-α-propionic acid. Similar treatment initiated growth in snapdragon ovaries but did not produce mature fruits. Phenylacetic acid caused seedless tomato fruits to develop and slight growth in snapdragons, but none in tobacco. Indoleacetic acid resulted in seedless fruits in the tomato, *Salpiglossis*, *Petunia*, *Begonia*, pepper, and eggplant. The same substance initiated ovarian growth in the snapdragon, *Zephyranthes carinata*, *Agapanthus umbellatus*, Crookneck Summer squash, and Hubbard squash, but not in cucumber and watermelon. Indolebutyric acid induced seedless fruits in tomato, *Salpiglossis*, *Petunia*, Crookneck Summer squash, *Begonia*, pepper, and eggplant but produced only slight growth in the ovaries of Hubbard squash, cucumber, and watermelon.

As a result of his own work and that of other investigators, GUSTAFSON (**8**) suggested that some fruits develop without fertilization because they have a higher auxin content in the ovary at the time of blossoming. He believed this content high enough to initiate growth processes, with the result that the ovaries commence to develop, even though there has been no fertilization. He found that the auxin content of ovules and developing seeds is much greater than that of other

parts of the fruit; moreover, the auxin content, in pepper at least, was much lower in the winter and spring than in the summer.

HAGEMANN (9) obtained parthenocarpic gladiolus by means of indoleacetic acid, but negative results from phenylacetic acid. GARDNER and MARTH (4) induced parthenocarpy in holly by spraying the open blossoms with naphthaleneacetic, indolebutyric, indoleacetic, and indolepropionic acids. The effectiveness of the substances followed the descending order as listed. They also found that better results were obtained by repeated applications. Moreover, parthenocarpic fruits were produced by watering the soil during full bloom with indoleacetic acid. They also obtained some parthenocarpic fruit by introducing small quantities of indoleacetic acid in powder form into holes in the stem made with a small nail. In a pistillate strawberry, parthenocarpic fruits of normal size but with empty achenes were induced by these workers, using the substances just mentioned in spray form. Negative results were obtained in the Starking apple from indoleacetic-acid spray and in the Brighton grape from naphthaleneacetic-acid treatment. In a further study, GARDNER and MARTH (5) found that naphthalene acetamide gave better results than naphthaleneacetic acid in inducing parthenocarpy in holly. Potassium-naphthalene acetate gave results far inferior to those obtained from phenylacetic acid and sodium-naphthol sulphonate. In studying the effect of certain growth substances on inflorescences of dates, NIXON and GARDNER (12) showed that 1 per cent naphthaleneacetic acid in lanolin paste applied to Thoory date arrested senescence of the perianth and strand. Negative results were obtained from indoleacetic and indolebutyric acids. In the Deglet Noor variety, spraying with naphthaleneacetic acid in aqueous solution about 10 days after pollination reduced the set by about 50 per cent.

Material and methods

This study was conducted at Michigan State College, East Lansing, during the summer of 1938 and the spring and summer of 1939. The varieties used were:

WATERMELON (*Citrullus vulgaris*): Winter Sweet, Fordhook Early, Early Arizona, Favorite Honey, Harris Earliest, Best Early, Select Early, Sweet Japanese, Tough Sweet, Northern Sweet (Selection nos. 1, 2, and 5), Coles Early, Early Kansas, Stone Mountain no. 5, Stone Mountain, Kleckley Sweet no. 6, Hawksbury, Iowa 1, Iowa 3, Iowa 5, "Yellow Melon," and six selected strains.

CUCUMBER (*Cucumis sativus*): National Pickling.

MUSKMELON (*Cucumis melo* var. *reticulatus*): An unnamed selection for the greenhouse tests in the spring 1939 and the variety Honey Rock for the summer experiments.

PUMPKINS AND SQUASHES (*Cucurbita pepo*): Early Prolific Straightneck, Dark Green Zucchini, Omaha, Delicata, Fort Berthol, White Bush Scallop, Table Queen, and Hardin Bush.

SQUASHES (*Curcurbita maxima*): Buttercup.

SQUASH (*Cucurbita moschata*): African Bell.

In addition, certain cucurbits of uncertain species carrying U.S.D.A. Plant Introduction nos. 127585, 127586, 127588, 127589, and 127590.

PEPPER (*Capsicum frutescens*): Harris Wonder.

TOMATO (*Lycopersicum esculentum*): Michigan State Forcing.

EGGPLANT (*Solanum melongena*): New Hampshire Hybrid.

STRAWBERRY (*Fragaria*): An unnamed variety of the everbearing type.

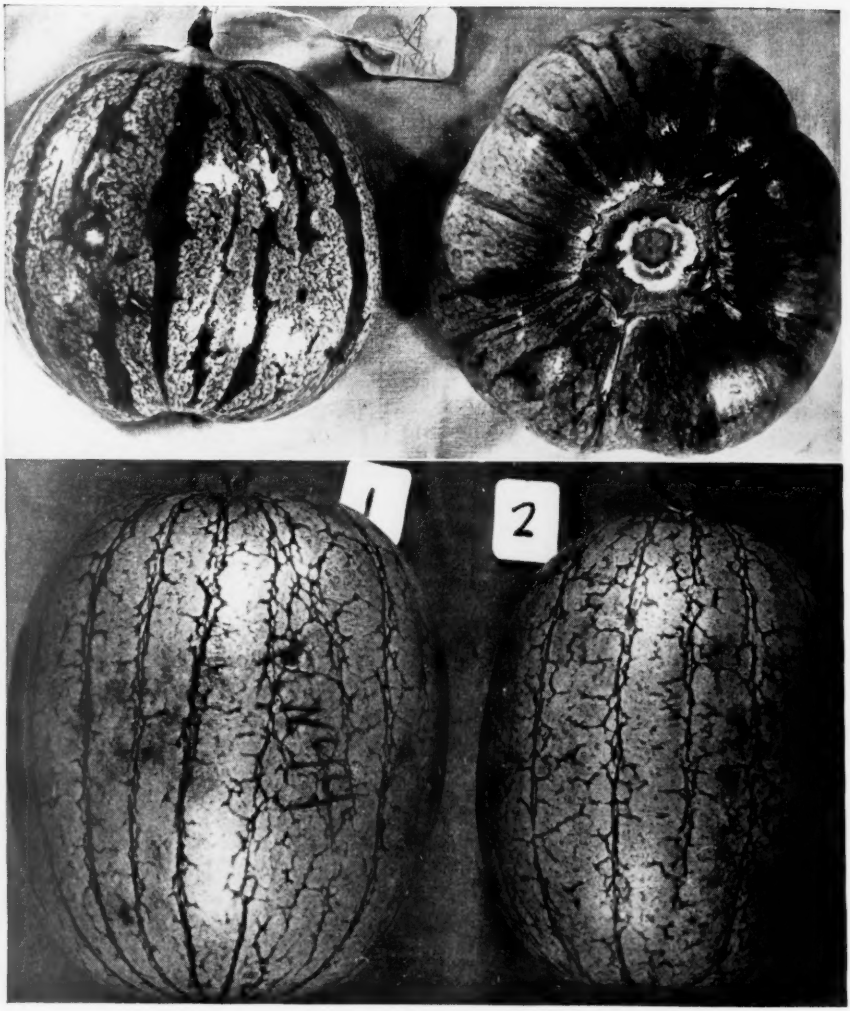
The Winter Sweet watermelon (fig. 1), also known as Dakota Sweet, is of medium size, red fleshed, weighing 9–14 pounds, with about 600 seeds per fruit. It is andromonoecious with occasional monoecious flowers. Northern Sweet is closely related to Winter Sweet, differing little either in external or internal characteristics. Favorite Honey (fig. 2) is a small, early, and very prolific melon with yellow flesh. It weighs 3–4 pounds and has about 250 seeds per fruit. The strains designated as Selection nos. 1–6 were derived from crosses between Favorite Honey and Winter Sweet. The seed of the Yellow Melon was purchased from a commercial firm as Favorite Honey, but later it was found to differ from Favorite Honey from other sources. This melon was much larger in size, averaging about 8–12 pounds, with very light lemon-yellow flesh. Its rind is exceptionally crisp and thin so that the fruits crack open easily. There are about 900 seeds per fruit, and the variety is andromonoecious. The sex condition of the other watermelon varieties was mostly monoecious, although occasionally hermaphrodite flowers were observed.

The synthetic growth substances used were naphthaleneacetic acid, potassium-naphthalene acetate, indolebutyric acid, colchicine, acenaphthene, sulfanilamide, and trimethylamine, mostly in lanolin paste. Naphthaleneacetic acid was also used as a spray by means of a hand atomizer.

The chemicals were thoroughly mixed with lanolin and these preparations smeared either on the stigma (if the style was short) or on the cut surface of the style (if the latter was long). If the style was more than 2 mm. it was found desirable to cut it off about 1 mm. above the ovary and smear the lanolin preparation on this surface.[2] Pastes prepared from growth substances were used in concentrations of 1, 2, 2.5, and 5 per cent. For spraying, 50 and 500 p.p.m. were used in the case of naphthaleneacetic acid and 40 p.p.m. in the case of trimethylamine. Since naphthaleneacetic acid is not soluble in water, the desired amount was first dissolved in a small quantity of alcohol, which was then added to the required amount of water.

The watermelons, cucumbers, muskmelons, eggplants, and peppers used in the field experiments were started in the greenhouse and transplanted to the field later. Some of the watermelon seeds were pre-treated with different chemicals, such as

[2] GUSTAFSON, F. G., Communication of March 5, 1937.



FIGS. 1, 2.—Fig. 1 (above), Winter Sweet watermelon: side and blossom-end view of parthenocarpic fruit produced by treating style with naphthaleneacetic acid in lanolin paste before anthesis. Note marked protruding stylar scar tissue in blossom-end portion. Fig. 2 (below), Favorite Honey watermelon: (*1*) from self-pollination with about 20 grains of pollen and with K-naphthalene acetate paste added; (*2*) from naturally pollinated pistil.

colchicine, acenaphthene, ether, and water, for various lengths of time before sowing.

Usually the flowers of each variety were subjected to four kinds of treatments: (1) pollinated in the usual way, (2) self-pollinated by hand, (3) treated with the substances under investigation, and (4) neither pollinated nor treated.

Flower buds nearly ready to open—but before anthesis had taken place—were selected. In hermaphroditic flowers, emasculation was carried out the day before treatment and the flowers protected with a wire cage. In a later experiment the style was cut off just above the ovary with a sharp scalpel, and the cut surface either smeared with the lanolin preparation or left without treatment as a control. Flowers so treated were not caged. A careful trial by GUSTAFSON (**6**), further confirmed by the writer, showed that if pollen is placed on the cut surface of the style there is never any development of the ovary to indicate that fertilization had taken place.

Investigation

EXPERIMENTS WITH WATERMELON

PRELIMINARY TRIALS (1938).—These included nine varieties and thirteen treatments. Pistils were not treated until after each plant had developed at least one rather good-sized fruit from self-pollination. Apparently this pollinated, seed-bearing fruit exerted some inhibiting effect on, or led to an unfavorable condition for, parthenocarpic development, thus explaining the poor results obtained (table 1).

No fruits were formed where emasculated flowers were left unpollinated or where the styles were cut and then lanolin paste, pollen, or indolebutyric acid applied to the cut surface. On the other hand, fruit development was induced by application of naphthaleneacetic acid, either to the stigmas or to the cut style (fig. 1). These watermelons were seedless and also lacked seed coats, with the exception of those of the variety Harris Earliest. They varied considerably in shape and size. In general they were slightly angular or even ribbed, although some were normal in shape and size. They were solid and firm in texture, and the flesh showed an intense red coloration. No difference in flavor could be detected when compared with normally pollinated fruits.

The three plants of the Winter Sweet watermelon grown from seed which had first been subjected to colchicine treatment showed a typical colchicine effect—stunting early in the season, large leaves and flowers, and great vigor later in the season. Although pollen was present in considerable abundance, it failed to induce fruit setting when the blossoms were selfed; on the other hand, hormone-treated flowers set satisfactorily.

In a parallel experiment involving colchicine pre-treated plants, a small fruit

was produced by self-pollination and a large one by treatment with 1 per cent naphthaleneacetic acid in lanolin paste. Both flowers were treated at the same time. The fruit from selfing dropped within 10 days, but the hormone-treated fruit continued to grow until maturity. Nevertheless, fruits containing apparently normal seeds were formed from some open-pollinated flowers (fig. 3*B*). Probably this was due to fertilization by nearby noncolchicine-treated plants. It is a case of vicinism. These fruits were much smaller in size and slower in growth than those from the hormone-treated blossoms.

TABLE 1

WATERMELONS: FRUIT SETTING AS RESULT OF HORMONE TREATMENT; 1938
DATA FOR NINE VARIETIES GROUPED TOGETHER

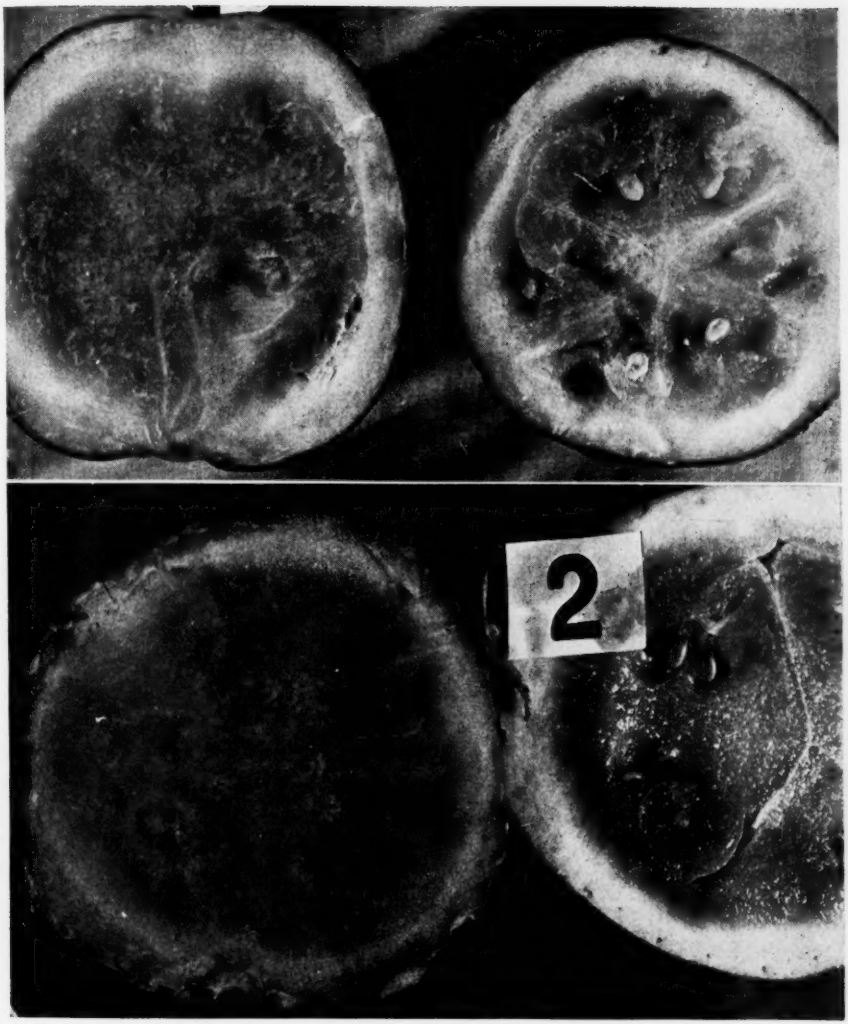
TREATMENT	NO. OF BLOSSOMS	NO. SET	PERCENTAGE SET	REMARKS
2.5% iba* in lanolin paste applied to cut style	42	0	0	
5% iba in lanolin paste applied to cut style	17	0	0	
2.5% iba paste applied to stigma	11	0	0	
Check, no treatment except emasculation	15	0	0	
Cut style only	7	0	0	
Pollen applied to cut style	7	0	0	
Lanolin paste applied to cut style	10	0	0	
2.5% naa paste applied to cut styles	10	8	80	Seven stopped growing at about $1\frac{1}{2}$ in. diameter
5% naa paste applied to cut style†	15	3	20	Fruits small and irregular shaped
2.5% naa paste applied to cut style†	12	4	33	
1% naa paste applied to cut style†	11	4	36	
Self-pollinated†	10	0	0	
Stigma sprayed with 0.05% naa solution†	2	2	100	Treated very late in season

* These abbreviations are used in this and the following tables: naa = naphthaleneacetic acid; kna = potassium-naphthalene acetate; iba = indolebutyric acid; a = acenaphthene; s = sulfanilamide; c = colchicine.

† Seeds pre-soaked in 0.4% colchicine aqueous solution for 4 days at room temperature.

GREENHOUSE TRIALS (1939).—About two dozen flower buds from Selection nos. 1, 2, and 5 were treated. Acenaphthene powder was first used to cover the cut styles, which were then covered with a lanolin paste containing 1 per cent naphthaleneacetic acid. Four fruits of normal size were ripened, 1900–3241 gm. in weight. They contained hard seed coats of about normal size but without embryos. It is a case of stenospermy. Three small fruits entirely lacking in edible flesh were also produced.

FIELD EXPERIMENTS (1939).—These included twenty-one varieties with sixteen treatments. The results are presented in table 2. Ovaries of flowers with treated stigmas or cut styles showed various responses. Some failed to develop at all;



FIGS. 3, 4.—Fig. 3 (above), Winter Sweet watermelon: left, longisection of naphthaleneacetic acid-treated fruit; right, cross section of open pollinated fruit. Note absence of seed coats in *A*. Seed from which plant was grown was soaked in 0.4 per cent colchicine solution for 4 days. Fig. 4 (below), Winter Sweet watermelon: two watermelons produced from naphthaleneacetic-acid treatment. The one without seeds and seed coats grew on a vine produced from seed pre-treated with colchicine; the fruit on the right produced what appeared to be seeds, but which were in reality undersized seed coats. Seed from which this plant was grown was given no such pre-treatment. Note greatly thickened rind in both cases.

others developed into small fruits which remained on the vine but never grew normally; others developed into normal fruits, except for the absence of seed.

Table 2 shows that a mixture of two growth substances gave better results than one alone. For example, when acenaphthene and naphthaleneacetic acid were used together, the percentage of parthenocarpic fruits produced was 7.2, as compared with 2.3 and 0 when naphthaleneacetic acid and acenaphthene, respectively, were

TABLE 2

WATERMELONS: FRUIT AND CROP SETTING AS RESULT OF APPLICATIONS OF HORMONES IN LANOLIN PASTE TO CUT STYLE; 1939

Treatment	No. of flowers treated	Fruit set*		Crop set*		No. of varieties used
		No.	%	No.	%	
1% naa	322	69	21.4	14	2.3	10
2% naa	402	149	37.1	29	7.2	13
1% kna	306	62	20.3	5	1.6	11
2% kna	309	97	31.4	16	5.2	12
1% each of naa and iba	301	87	28.9	15	5.0	12
1% naa plus 10% a	430	143	33.3	31	7.2	10
1% kna plus 0.1% c	152	46	30.3	4	2.6	8
1% kna plus 0.5% c	106	46	43.4	15	14.2	7
1% kna, 1% a, 1% iba, and 0.1% c	101	39	38.6	9	8.9	6
About 20 grains of pollen applied to stigma	103	2	1.9	1	1.0	5
About 20 grains of pollen applied to stigma plus:						
1% kna paste to stigma	102	22	21.6	3	2.9	7
1.5% s	50	0	0	0	.....	.....
10% a	84	0	0	0	.....	.....
Check, cut style only	524	0	0	0	.....	13
Selfing of one-lobed stigma and removing of other two lobes	29	2	6.9	1	3.5	5
Self-pollinated	180	80	44.4	66	36.7	.....

* The terms fruit set and crop set in this and following tables are used to describe the degree of development obtained: fruit set applies to fruits 1.5–12.5 cm. in diameter, while crop set applies to fruits more than 12.5 cm. in diameter.

used alone. Indolebutyric acid failed to induce parthenocarpic development at concentrations of 1, 2.5, and 5 per cent but when a mixture of 1 per cent each of indolebutyric and naphthaleneacetic acids was used, 5 per cent of parthenocarpic fruits of normal size were obtained.

In these experiments a concentration of 2 per cent of the different chemicals gave better results than 1 per cent. There was some indication that the naphthaleneacetic acid had a greater influence on parthenocarpic development than its potassium salt.

To determine whether a watermelon fruit can develop with a very limited number of seeds, about twenty grains of pollen were applied to the stigmas of each of a number of flowers. Only one fruit developed from the 103 flowers treated in this way. By adding a growth substance (K-naphthalene acetate) to the stigma, how-

ever, followed by application of a limited amount of pollen, three mature fruits were obtained from 102 treated flowers. In general, all the hormone-treated ovaries that did not develop into mature fruits enlarged to several times their original size, then ceased further growth, turned soft, and dried but persisted on the vine, even when the plants were killed by the first frost. On the contrary, the untreated and unpollinated ovaries were shed within 7–10 days.

TABLE 3

WATERMELONS: RESPONSE TO THE VARIOUS HORMONE TREATMENTS LISTED IN TABLE 2*

Variety	No. of flowers treated	Fruit set		Crop set	
		No.	%	No.	%
Winter Sweet	567	187	32.9	31	5.5
Northern Sweet	540	96	17.7	2	0.4
Favorite Honey	204	69	33.8	40	19.6
Coles Early	64	33	51.5	3	4.7
Early Kansas	45	10	22.2	1	2.2
Fordhook Early	94	30	31.9	2	2.1
Stone Mountain	62	26	41.9	2	3.2
Kleckley Sweet	34	15	44.1	0	.....
Hawksbury	83	15	18.0	1	1.2
Iowa 5	26	10	38.4	1	3.8
Selection 1	45	0	.....	0	.....
Selection 2	11	5	45.4	5	45.5
Selection 3	2	0	.....	.....	.....
Selection 4	11	2	18.1	.....	.....
Selection 5	58	10	17.2	4	6.9
Harris Earliest	10	2	20.0	0	.....
Yellow Melon	565	226	40.0	46	8.1

* With the exception of pollination, check and treatments with acenaphthene and sulfanilamide.

Colchicine, when mixed with the synthetic growth-substance preparation, gave an increase in the percentage of parthenocarpic fruits from any treatment: a 14.2 per cent set resulted from a mixture of 1 per cent naphthaleneacetic acid and 0.5 per cent colchicine preparation. Probably colchicine may be considered as a growth substance from the standpoint of parthenocarpic development.

By removing two-thirds of the stigmatic surface and pollinating only the remaining portion, normal seed-bearing fruits were produced.

Differences in varietal response to various hormone treatments are presented in table 3.

Table 3 shows that varieties differ greatly in their ability to produce parthenocarpic fruit as a result of treatment. For instance, Favorite Honey and Yellow Melon gave high percentages of parthenocarpic fruits, but Early Kansas, Northern Sweet, and Selections 1, 3, and 4 produced none or very few. Northern Sweet is

probably closely related to Winter Sweet; however, the former variety gave a much lower percentage of parthenocarpy. Moreover, Selections 1–5 were all derived from a cross between Winter Sweet and Favorite Honey, but only Selections 3 and 5 produced parthenocarpic fruits.

Table 4 presents the responses of four representative varieties to the various hormone treatments. The responses agree closely with those shown in table 2.

TABLE 4

WATERMELONS: NUMBERS AND PERCENTAGES OF FRUIT AND CROP SETTING WITH HORMONES IN LANOLIN PASTE APPLIED TO CUT STYLES

Treatment	Winter Sweet			Northern Sweet			Favorite Honey			Yellow Melon		
	A*	B*	C*	A	B	C	A	B	C	A	B	C
1% naa	85	24.7	2.4	95	5.3	0	25	16.0	12.0	70	40.0	7.1
2% naa	64	37.5	6.3	120	29.2	0	37	48.6	35.2	98	39.7	7.1
1% kna	39	20.5	2.6	66	6.1	0	11	27.3	18.2	102	29.4	1.0
2% kna	27	7.4	0	78	16.7	1.3	22	27.3	13.6	55	49.1	12.7
1% mixture of naa and iba	90	30.0	4.4	61	16.4	0	28	32.1	17.9	74	39.2	5.4
1% naa plus 10% a	181	43.1	8.3	52	17.3	1.9	10	10.0	10.0	96	38.5	29.7
1% kna plus 0.1% c	26	50.0	3.8	43	23.3	0	22	22.7	4.5	15	60.0	13.3
1% kna plus 0.5% c	24	16.7	0	18	33.3	0	49	46.9	24.5	11	90.9	27.3
1% each of kna, a, iba, and 0.1% c	31	32.3	3.2	7	57.1	0	.....	.....	.....	44	38.6	13.6
About 20 grains of pollen applied to stigma	37	0	0	32	0	0	12	0	.....	19	10.5	5.3
About 20 grains of pollen applied to stigma plus 1% kna paste to stigma	32	25.0	0	15	26.7	0	15	20.0	20.0	35	17.1	0
1.5% s	14	0	.....	7	0	.....	10	0	.....	15	0	.....
10% a	51	0	.....	20	0	.....	.....	.....	.....	.....	.....	.....
Check, cut style only	50	0	.....	48	0	.....	78	0	.....	207	0	.....
Self-pollination by hand	26	57.7	53.8	32	15.6	15.6	10	70.0	70.0	41	53.6	46.3

* A, number of flower treated; B, percentage of fruit setting; C, percentage of crop setting.

For instance, naphthaleneacetic acid gave a better set than its potassium salt, and a mixture gave more favorable results than one hormone alone. Favorite Honey gave the highest set with all treatments, but no initial development was noted when a very limited amount of pollen was applied. When pollen was added with a growth substance, however, fairly good results were obtained.

No initial growth was observed on the check flowers of any variety. If natural parthenocarpy could occur, it might be expected on Favorite Honey or Yellow Melon, which gave the highest percentage of stimulative parthenocarpy.

The varietal responses of watermelons to various treatments with respect to fruit size, shape index, and weight are presented in table 5. Although the means

TABLE 5

WATERMELONS: MEAN FRUIT SIZE, SHAPE INDEX, AND WEIGHT AS RESULT OF TREATMENT

Variety	Treatment	No. of fruits	Diameter (cm.)		Shape index*	Weight (gm.)
			Polar	Equatorial		
Winter Sweet	1% naa	5	16.3	13.4	0.82	1551
	2% naa	4	16.3	14.3	0.88	1481
	1% kna	1	14.1	13.6	0.97	1205
	1% naa and 1% iba	4	17.2	14.0	0.80	1653
	1% naa, 10% a	14	15.9	13.7	0.86	1452
	1% naa, 0.1% c	1	21.0	16.9	0.80	2821
	1% each of kna, a, and iba; 0.1% c	1	25.1	15.1	0.60	2600
	1.5% s	6	19.9	19.5	0.98	3169
Northern Sweet	2% kna	1	15.1	11.0	0.73	857
	1% naa, 10% a	1	23.1	16.6	0.72	3070
	1.5% s	8	22.4	20.9	0.93	4195
Favorite Honey	1% naa	3	13.9	13.0	0.93	1133
	2% naa	13	14.7	12.1	0.82	1089
	1% kna	2	16.5	12.9	0.78	1455
	2% kna	3	13.8	11.1	0.81	983
	1% naa, 1% iba	5	19.0	14.0	0.74	1628
	1% naa, 10% a	1	15.0	12.0	0.80	1300
	1% naa, 0.1% c	1	12.6	10.3	0.82	733
	5% naa, 0.1% c	10	14.2	12.3	0.87	1158
	About 20 pollen grains; 1% kna	3	22.3	15.3	0.69	2374
	1.5% s	2	18.3	15.8	0.86	2062
Coles Early	2% naa	1	18.9	15.1	0.80	2080
	2% naa, 10% a	2	23.0	18.0	0.78	3521
Early Kansas	2% kna	1	17.5	12.0	0.69	920
Fordhook Early	1% kna	1	21.4	19.2	0.90	3972
	1% naa, 10% a	1	19.2	13.8	0.72	1810
Stone Mountain	1% each of kna, a, and iba; 0.1% c	2	14.9	12.3	0.83	533
Hawksbury	2% naa	1	23.2	12.8	0.55	1679
Iowa 5	2% kna	1	24.0	19.4	0.81	4008
Selection 2	2% naa	2	19.9	17.1	0.86	2379
	2% kna	2	15.6	15.6	1.00	1893
	1% naa, 10% a	1	13.8	12.1	0.88	894
	1.5% s	1	21.2	21.0	0.99	3850
Selection 5	1% naa	1	16.0	13.5	0.84	.....
	2% naa	1	15.0	11.0	0.73	1008
	2% kna	1	14.6	12.6	0.86	1023
	1% naa, 10% a	1	13.8	11.8	0.86	1034
Yellow Melon	1% naa	5	21.2	19.4	0.91	3603
	2% naa	6	18.4	16.5	0.90	2631
	1% kna	1	15.6	13.6	0.87	1439
	2% kna	7	21.4	20.0	0.94	3512
	1% naa, 1% iba	4	20.0	16.8	0.84	2535
	1% naa, 10% a	10	18.0	16.1	0.90	2617
	1% naa, 0.1% c	2	18.6	18.2	0.98	3142
	5% naa, 0.1% c	3	16.9	15.1	0.90	1985
	1% each of kna, a, and iba; 0.1% c	6	15.4	14.3	0.92	1701
	About 20 pollen grains	1	15.1	11.8	0.78	959
	Self pollinated	12	21.1	19.9	0.95	4048

* Shape index = $\frac{\text{Equatorial diameter}}{\text{Polar diameter}}$.

represent only a limited number of fruit measurements, they indicate that the average size of the parthenocarpic fruit was less than that of pollinated seed-bearing fruits. However, parthenocarpic fruits of normal size or even larger than those from pollination were obtained in some varieties, such as Favorite Honey and Yellow Melon, when their ovaries were treated with certain growth substances. Moreover, many fruits of normal shape were produced as a result of parthenocarpic development, as indicated by the shape indices.

Most of the parthenocarpic fruits that developed to full size were normal in appearance, except that they lacked seeds. On the other hand, some fruits which can be induced to set parthenocarpically do not respond normally to treatment, as

TABLE 6

WATERMELONS: CHARACTERISTICS OF PARTHENOCARPIC AND POLLINATED SEED-BEARING FRUITS

VARIETY	TREATMENT	THICKNESS OF RIND (MM.)	COLOR OF FLESH*	HOLLOWNESS	STYLAR SCAR TISSUE
Winter Sweet....	P†	9.5–4.7	Red (Vermilion 18/2)	Hollow	Sunken
	H	15.9–8.6	Deep red (Signal Red 719/2)	Solid or hollow	Protruding
Yellow Melon...	P	9.2–4.3	Pale yellow (Aureolin 3/3)	Hollow	Sunken
	H	12.8–7.0	Orange yellow (Lemon Yellow 4/1 to Tangerine Yellow 9/3)	Mostly solid	Protruding

* Color description based on Horticultural Color Chart (1).

† P = pollination; H = hormone.

indicated by abnormal development of their various tissues. Their flesh (placentae) is lacking or only partially developed, or a light margin may occur in the place of contact between placental regions; there is often an increase in the thickness of rind, and intense coloration of flesh. The fruits may be slightly triangular or even ribbed. Comparisons between self-pollinated and parthenocarpic watermelons from all hormone treatments, with respect to rind thickness, flesh color, hollowness, and stylar scar tissue, are presented in table 6.

The rind of the hormone-treated fruits was almost twice as thick as that of the pollinated ones. The coloration was more intense in the parthenocarpic fruits. The texture was firm and less juicy, especially in the Winter Sweet variety, when the seeds were pre-treated with colchicine (fig. 4). Another outstanding difference between them was their stylar scar tissue, which protruded in the fruits which developed from applying the growth substance to the cut style (fig. 1).

The varietal responses with respect to the size and weight of seeds and seed coats as well as color and texture of seed coats from self-pollinated and parthenocarpic fruits in watermelons are presented in table 7 and figure 5. These data

show that no seed was developed in any of the treated fruits, although seed coats were developed to various degrees. Winter Sweet, Northern Sweet, Favorite

TABLE 7

WATERMELONS: VARIETAL RESPONSE IN SEED AND SEED-COAT DEVELOPMENT AS SHOWN BY SELF-POLLINATED AND PARTHENOCARPIC FRUITS

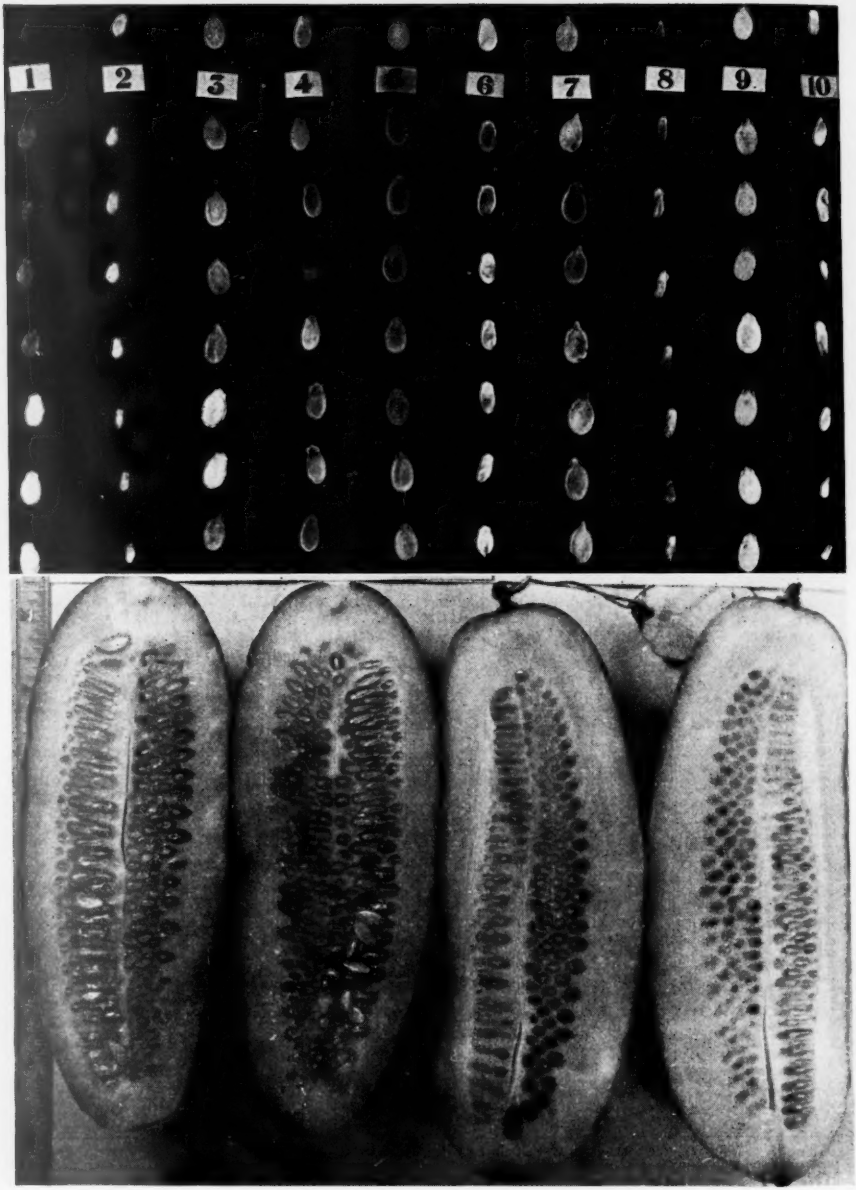
Variety	Treatment	Seed		Seed coat		
		Size (mm.)	Weight* (gm.)	Size (mm.)	Weight (gm.)	Appearance
Winter Sweet	P	12.1×7.2	7.744			Black, hard
	H†	o		10.0×6.0	1.562	Black, hard
	H‡	o		o		
Northern Sweet	P	12.0×7.4	7.350			Yellow, hard
	H	o		10.0×6.0	1.556	
Favorite Honey	P	11.0×7.2	5.040			Brown, hard
	H	o		9.8×6.2	2.075	Brown, hard
	P+H	10.8×7.2		9.3×5.7	1.490	Brown, hard
Coles Early	P	13.5×8.5	9.025			Black, hard
	H	o		10.4×5.5	1.345	Light yellow, soft
Early Kansas	P	14.0×8.8	12.120			Brown, hard
	H	o		14.0×5.8	1.100	Yellow, papery
Fordhook Early	P	13.0×8.0	10.105			Light yellow, hard
	H	o		9.2×5.4	0.830	Light yellow, papery
Iowa 5	P	13.5×8.0	9.120			Light yellow, hard
	H	o		8.5×5.0	0.708	Light yellow, papery
Selection 2	P	12.2×8.0	10.517			Black, hard
	H	o		10.2×6.4	1.725	Black, hard
Selection 5	P	o				
	H	o		9.8×5.9	1.178	Light brown to semi-hard
Stone Mountain	P	13.8×8.5	11.352			Yellow, hard
	H	o		8.7×4.9	0.300	Yellow, papery
Hawksbury	P	15.5×7.0	8.000			Black, hard
	H	o		8.0×4.0	0.275	Brown, papery
Yellow Melon	P	9.6×6.3	5.283			Dark brown, hard
	H	o		6.3×3.8	0.342	Light yellow, papery
Early Arizona	P	12.3×8.1	12.140			Black, hard
	H	o				

* Weights of seeds and seed coats calculated on air-dry condition and given in grams per 100 seeds from composite sample of 100–500 individuals.

† Composite sample of all treatments, of different concentrations and combinations.

‡ Seeds from these plants had been soaked in a 0.4 per cent colchicine aqueous solution.

Honey, and Selection 2 produced what appeared to be normal seeds; however, none possessed an embryo. Yellow Melon, Fordhook Early, and Stone Mountain



FIGS. 5, 6.—Fig. 5 (above), watermelon seeds: normal seeds from self-pollination as indicated in *1, 3, 5, 7* and seed coats from hormone treatment; *2, 4, 6, 8* in various varieties of watermelons listed below: *1, 2*, Yellow Melon; *3, 4*, Winter Sweet; *5, 6*, Fordhook Early; *7, 8*, Stone Mountain; *9, 10*, Iowa 5. Note only empty seed coats, except for different degrees of development, in hormone-treated fruits. Fig. 6 (below), National Pickling cucumber: longitudinal sections of typical naphthaleneacetic acid-treated cucumber (at left) showing "undeveloped seeds," compared in shape and size with open-pollinated cucumber (at right).

possessed only very small, papery seed coats. Pre-treatment of the seeds with a proper dosage (0.4 per cent for 4 days in Winter Sweet in this case) of colchicine prevented development of the integuments into seed coats in the parthenocarpic fruits. On the other hand, neither mixing the colchicine powder with the growth substance (K-naphthalene acetate) in the paste applied to the cut style of the blossom nor applying the substance to the flower produced from a stem which has been painted with colchicine paste prevented seed-coat development. Pre-treatment of the seeds with acenaphthene and other chemicals did not prevent development of empty seed coats.

Experiments with cucumber

In the American type cucumber, such as the National Pickling variety, a constriction of the stem end or blossom end is usually associated with seedlessness of

TABLE 8

National Pickling cucumber: Fruit setting as result of hormone treatment; 1938

Treatment	No. of blossoms	No. set	Percentage set
2.5% naa in lanolin paste applied to cut style cap	30	13	43
1% naa in lanolin paste applied to cut style cap	32	24	75
5% naa in lanolin paste applied to cut style cap	25	5	25
No treatment except nipped-off stigma	30	3	10
No treatment and no pollination	44	5	11
1% naa paste applied to stigma	11	6	54
Self-pollinated	15	8	53
Female flower sprayed with 0.05% naa solution with hand atomizer*	42	14	33

* This treatment used near end of season, which may account for low percentage of set.

that particular portion. Vegetative parthenocarpy seldom occurs in this variety (**13, 14**). An experiment was conducted with this variety in an attempt to secure seedless fruits of normal straight shape by means of growth substances. There were eight series of treatments. The treated blossoms were covered with wire cages before anthesis and left covered for 4–6 days after treatment. Naphthaleneacetic acid was the only hormone used. The results are given in table 8.

Although the number of samples was not large enough to permit any conclusive statement, the results indicate that naphthaleneacetic acid did induce parthenocarpic fruits of normal size and shape, either when the hormone was applied in lanolin paste of 1–5 per cent concentration or as a 0.5 per cent aqueous solution. Longitudinal sections of a self-pollinated seed-bearing cucumber and a stimulative parthenocarpic fruit are shown in figure 6. Hard viable seeds were present in the pollinated fruits; only soft, small, and undeveloped "ovules" were found in the parthenocarpic ones.

In the spring of 1939, under greenhouse conditions, about one dozen flower buds of the same variety were used in a further test. After the stigma and corolla were removed, the style cap was sprayed with naphthaleneacetic acid and trimethylamine in concentrations of 500 and 40 p.p.m., respectively. Three of the flowers were treated with 1 per cent naphthaleneacetic-acid paste. Straight fruits of normal size, having undersized seed coats without embryos, were obtained. One fruit which was produced by spraying with naphthaleneacetic acid at a concentration of 500 p.p.m. on April 26 was picked on June 19. It weighed 1132 gm. and was 26.5 cm. in length.

Experiments with Cucurbita pepo

In the spring of 1939 an unnamed pumpkin of this species was used in the greenhouse. Four flowers were treated with acenaphthene and 1 per cent naphthaleneacetic-acid paste applied to the cut style on May 3. Three "fruits" reached the size of 5.0×4.9 cm., then turned soft and finally dropped on May 16. The two check flowers dropped when their ovaries had attained a size of 1.8×2.0 cm.

TABLE 9

Early Prolific Straightneck squash: Fruit and crop setting as result of hormone treatment in lanolin paste to cut styles

Treatment	No. of flowers treated	Fruit set		Crop set	
		No.	%	No.	%
1% kna	3	3	100	3	100
2% kna*	10	10	100	.....	.....
1% kna and 0.5% c	4	1	25	1	25
Check, cut style only	2	0	0	.....	.....

* Blossoms treated very late in season and killed by first frost.

In the summer of 1939, eight varieties were used in further tests. The results with Early Prolific Straightneck and Dark Green Zucchini are shown in tables 9 and 10. The flowers were limited in number, but they indicated that both varieties are susceptible to parthenocarpic development. No external differences could be detected between normal and parthenocarpic fruits of squash. Although these two varieties belong to the same species, *Cucurbita pepo*, the integuments did not undergo the same degree of development in their parthenocarpic fruits. There were small, soft-textured seed coats in Early Prolific Straightneck but none in Dark Green Zucchini.

Other varieties used in this experiment, but with a more limited number of flowers (10–20 blossoms), were: Top of the Market, Hardin Bush, Omaha, Deli-

cata, Table Queen, and Fort Barthol. One mature fruit of Top of the Market was obtained by treatment with 2 per cent K-naphthalene acetate paste. No seeds or empty seed coats were found. One fruit of Hardin Bush was obtained from the same hormone treatment but dropped when it reached the size of 12.5×11.0 cm. Very little growth was observed in the treated ovaries of Fort Berthol, Omaha, and Delicata.

Experiments with Cucurbita maxima

In the spring of 1939, Buttercup squash was grown in the greenhouse. The cut styles of a few flower buds were treated with acenaphthene, followed by 1 per cent naphthaleneacetic acid paste, and another group was treated with naphthalene-

TABLE 10

Dark Green Zucchini squash: Fruit and crop setting as result of hormone treatment in lanolin paste to cut styles

Treatment	No. of flowers treated	Fruit set		Crop set	
		No.	%	No.	%
1% kna	5	2	40	2	40
2% kna*	8	7	87	.....	.....
1% naa and 10% a	2	1	50	0	.....
1% kna and 0.5% c	2	1	50	1	50
1% kna, 1% a, 1% iba, and 0.1% c	10	4	40	1	50
Check	2	0	0	.....	.....

* Blossoms treated very late in season and killed by first frost.

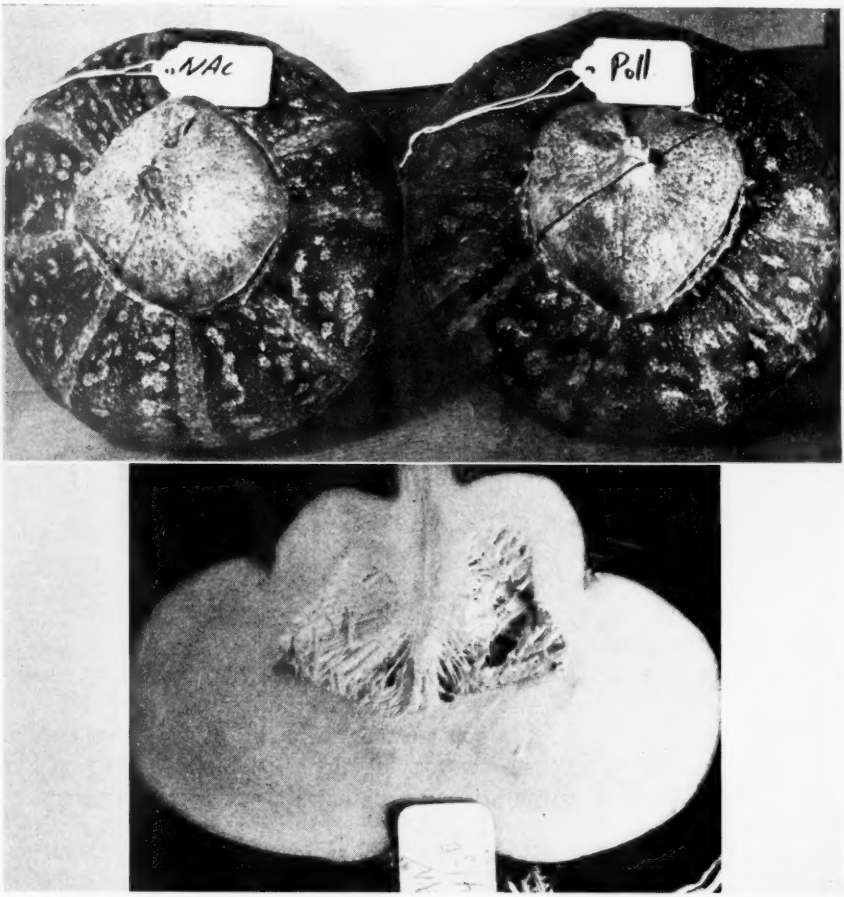
acetic acid alone. Flowers were treated on April 30 and the fruits matured about June 10. Seven externally normal fruits were produced (fig. 7), which were without seeds (fig. 8). The placental region of the parthenocarpic fruits was more restricted and they had a much thicker receptacle and pericarp than had normal seed-bearing fruits. Stylar tissue was fully developed. One undersized fruit was produced from treatment with 1 per cent naphthaleneacetic acid. In the summer of 1939 further experiments with the same variety were conducted. The results are shown in table 11.

Experiments with Cucurbita moschata

In the spring of 1939, the African Bell variety was used in the greenhouse. Out of four flowers treated with acenaphthene and 1 per cent naphthaleneacetic-acid paste, three normal fruits were obtained. One good fruit was also produced from naphthaleneacetic-acid treatment. The flowers were treated May 8 and fruits were picked July 6. One of them weighed 3270 gm. Fairly large seed coats, soft in texture, were present.

Experiments with Cucumis melo var. reticulatus

In the spring of 1939 about one dozen flowers of an unnamed selection of muskmelon were used and three mature fruits secured. In the summer of 1939, thirty-



Figs. 7, 8.—Fig. 7 (above), Buttercup squash: blossom-end view of hormone-treated fruit (*nac*) and pollinated fruit (*poll*) showing similarity in external appearance. Fig. 8 (below), Buttercup squash: longisection of hormone-treated fruit showing absence of seeds and seed coats. Placental region more restricted and with much thicker receptacle and pericarp than normal seed-bearing fruit.

five flowers of Honey Rock were treated with 1 and 2 per cent each of naphthaleneacetic acid and its potassium salt and a mixture of 1 per cent naphthaleneacetic acid and 10 per cent acenaphthene. Only negative results were obtained. A

limited number of flowers of cucurbits of unidentified species carrying U.S.D.A. Plant Introduction numbers were treated with various growth substances, but no parthenocarpic fruits developed.

Experiments with solanaceous plants

Pepper (Capsicum annum).—In the summer of 1938, various tests were made on the Harris Wonder variety. Twenty-four fruits out of twenty-five blossoms developed parthenocarpically as a result of treatment with 1 per cent naphthaleneacetic acid. Four fruits developed following spraying the stigmas with 0.05

TABLE 11

Buttercup squash: Fruit and crop setting as result of hormone treatment in lanolin paste to cut styles

Treatment	No. of flowers treated	Fruit set		Crop set*	
		No.	%	No.	%
1% kna	12	9	75	5	42
1% each of naa and iba	7	1	14	0	.....
1% naa and 10% a	9	2	22	0	.....
1% naa and 10% a†	10	9	90	9	90
1% kna and 0.1% c	11	9	82	7	64
1% kna and 0.5% c	7	3	43	1	14
1% kna, 1% a, 1% iba, and 0.1% c	10	5	50	1	10
Check, cut style only	7	0	0	0	.....

* By criteria of (*a*) loss of semi-glossy green color in rind and presence of yellow color near ground portion, (*b*) full size, and (*c*) toughness of rind.

† Application of abundance of acenaphthene to the cut style, then covering with naphthaleneacetic-acid paste. Treatment conducted in the spring of 1939 in the greenhouse.

per cent naphthaleneacetic acid. Out of seven flowers, the styles of which were cut but given no further treatment, four formed fruits which were seedless. Possibly parthenocarpy in this instance may have been due to the stimulating effect of some wound hormone from the cut style or to vegetative parthenocarpy. The treatment was started very late in the season, and all the plants were killed by frost before the fruits reached full maturity; however, all partly grown, hormone-treated fruits were seedless and normal in shape.

Tomato (Lycopersicum esculentum).—Fifteen flowers of the Michigan State Forcing variety were emasculated and treated with naphthaleneacetic acid. The results are presented in table 12. No seed developed. Most of the fruits were solid in the placental region.

Fruits resulting from an aqueous spray were much smaller than those produced from the paste treatment. Many of the parthenocarpic fruits showed internal breakdown at the blossom end, with the appearance of blossom-end rot.

EGGPLANT (SOLANUM MELONGENA).—Flowers of the variety New Hampshire Hybrid were treated late in the summer of 1939, and the fruits were still small when picked because of frost. The largest of the six parthenocarpic fruits reached a size of 11.5×10.2 cm. from treatment with a mixture of 1 per cent naphthaleneacetic acid and 10 per cent acenaphthene. Fruits from treatment with a mixture of 1 per cent each of naphthaleneacetic and indolebutyric acids were somewhat smaller. There appeared to be no external differences between pollinated and hormone-induced fruits, although the normally pollinated fruits grew more rapidly.

TABLE 12

TOMATO (MICHIGAN STATE FORCING): FRUIT SETTING AS RESULT OF HORMONE TREATMENT

TREATMENT	NO. OF FLOWERS TREATED	NO. OF FRUITS SET	RANGE IN WEIGHT GM.)
Sprayed with 500 p.p.m. naa solution..	9	9	33– 69
Sprayed with 50 p.p.m. naa solution...	3	3	42– 60
1% naa paste applied to cut style.....	3	3	133–150

TABLE 13

STRAWBERRY: FRUIT SETTING AS RESULT OF HORMONE TREATMENT

TREATMENT	NO. OF FLOWERS TREATED	NO. OF FRUITS SET	REMARKS
Sprayed with 500 p.p.m. naa..	12	12	Average over 20 mm. diameter, achenes small and embryoless
Self hand-pollination.....	1	1	26 mm. diameter, achenes large with embryos
Open pollination.....	5	2	22 mm. diameter, achenes without embryos
Check, emasculation only.....	4	1	14 mm., lop-sided

On sectioning, only minute lines showed where the locules would normally have been, and there were no signs of ovules. The one control dropped within a week after the style was cut off.

EXPERIMENTS WITH STRAWBERRY

In the late fall of 1938, potted plants of an unnamed ever-bearing strawberry were used for fruit-setting experiments. About two dozen flower buds were treated. Unopened hermaphrodite flowers were first emasculated, then sprayed with naphthaleneacetic acid in concentrations of 50 and 500 p.p.m. with a hand atomizer. Parthenocarpic fruits of normal size containing achenes that appeared

normal were obtained; however, the latter were found to lack embryos. Some of the treated receptacles made only a slight initial growth, and achenes rarely developed on them. In the spring of 1939, a further experiment was conducted, the results of which are presented in table 13.

Summary

1. No parthenocarpy, vegetative or stimulative, other than that due to hormone treatment, occurred in the watermelons and other species of *Cucurbita* tested, except for cucumber and pepper.

2. A very limited number of pollen grains did not cause the watermelon to set fruit. By adding a growth substance, however, mature fruits were obtained.

3. Treated watermelon flowers which did not develop and mature parthenocarpic fruits yet persisted on the vine, apparently because the substances prevented the formation of an abscission layer.

4. No apomictic seed of any sort developed in the parthenocarpic fruits produced from growth substances.

5. A mixture of two growth substances gave better results than one alone; for example, indolebutyric acid, acenaphthene, and sulfanilamide induced parthenocarpy when employed alone, but gave more favorable results when mixed with other substances.

6. Indolebutyric acid (1–5 per cent), acenaphthene (10 per cent), and sulfanilamide (1.5 per cent) in lanolin paste failed to induce parthenocarpic development in watermelon.

7. Naphthaleneacetic acid had a greater effect on parthenocarpic development than its potassium salt and other growth substances.

8. There were great variations in fruit size and seed-coat development among the parthenocarpic fruits. Normal-sized hard seed coats were occasionally present in some fruits; however, seedless fruits of normal size which lacked seed coats were present in many species or certain varieties within the same horticultural group.

9. Pre-treatment of watermelon seeds with proper dosage of colchicine prevented development of integuments into seed coats on parthenocarpic fruits. Mixing the colchicine with a growth substance in the paste applied to the cut style did not prevent such seed-coat development.

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HYPERAUXINY IN CROWN GALL OF TOMATO[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 526

GEORGE K. K. LINK AND VIRGINIA EGGERS

(WITH ONE FIGURE)

Introduction

The literature contains several reports bearing on the relative auxin contents of healthy organs of tomato and of the same organs bearing galls incited by inoculation with the schizomycete *Phytomonas tumefaciens* (**14, 16, 17, 18, 26, 27**).

LOCKE, RIKER, and DUGGAR (**17**) compared noninoculated and inoculated plants, including tomato, as to epinasty of leaf petioles, initiation of adventitious roots, stimulation of cambium, inhibition of bud development, and delay of petiole abscission. They also compared the intensity of these responses following inoculation with avirulent and virulent strains of the parasite. It was concluded that these responses indicated an increase in growth substances in the inoculated plant and that it is less marked in those inoculated with the avirulent strain.

LINK, WILCOX, and LINK (**14**) prepared chloroform extracts of galls incited in tomato following inoculation with *P. tumefaciens*, and of control stems and leaves of tomato, and applied these to different parts of the axis of red kidney bean and tomato. Application of the gall extract through puncture in the bean hypocotyl was followed by whitening and local swelling. Application to the second internodes was followed by slight swelling and slight positive curvature. Extracts of control stems and leaves produced no effects. Application of the gall extract to hypocotyls of tomato seedlings led to slight negative bending and often to killing. In older tomato plants only killing was noted.

LOCKE, RIKER, and DUGGAR (**18**) used diffusion and the *Avena* technique (**33**) in testing the auxin content of noninoculated seedlings of tomato and of seedlings inoculated with *P. tumefaciens*. They made tests on the third, sixth, and ninth days after inoculation, using only one seedling of each kind on each of these days. On the basis of these limited tests they reported that the inoculated plants diffused more growth substance than the controls and that the differences increased with time. They also applied the diffusion technique to well-developed crown galls but could find no significant amount of auxin. They likewise failed to obtain any auxin when they assayed the chloroform and alcohol extracts of large galls.

Later RIKER, BERCH, and DUGGAR (**27**) reported—on the basis of extensive

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diffusion and extraction tests with segments of tomato plants bearing crown gall and with control segments of the same plant—that no significant differences in auxin contents exist between inoculated and control tissues on 1, 4, 8, and 16 days after inoculation. They conclude: "crown gall and control tissues are similar in relation to auxin obtained by extraction and diffusion."

LINK, EGGERS, and MOULTON (**16**), in reporting on the preparation of plant tissues for auxin analysis by freezing and drying in vacuum and on success in demonstrating an auxinically more active extract from nodules of bean than from its roots, mentioned in passing that by use of material so prepared extracts of galls of tomato give a higher auxin assay than extracts of the control organ.

This paper reports the results of *Avena* coleoptile tests of extracts of noninoculated hypocotyls of tomato and of hypocotyls of the same age and cultural conditions but bearing large crown galls produced by inoculation with pure cultures of *P. tumefaciens*. The first part of the paper is devoted to a comparison of extractants and of extraction procedures; the second, to comparative assays of noninoculated and inoculated organs.

Material and methods

Seed of tomato, variety Bonnie Best, was sown in soil in pots on September 17, 1940. Plants were thinned and selected for vigor and uniformity. On October 15, one half of the 786 plants available were set aside as controls, and the other half were inoculated in the hypocotyl with a pure culture of *Phytomonas tumefaciens* (Smith and Town.) Bergey *et al.* At this stage of development the hypocotyl had almost, but not completely, ceased elongation; each plant had two expanded leaves, and the internodes were beginning to elongate. The hypocotyl was selected for assay because it is the first organ to complete its elongation, and because its length and diameter are remarkably uniform in a population of plants grown under identical cultural conditions. The hypocotyls were inoculated with a special instrument, a micro-trident, with needles 4 mm. long and 1 mm. apart set firmly in a row. This insured that the triple puncture of each plant was identical as to spacing and depth. Before the puncture was made the inoculum was placed on the site of inoculation, which was midway between the ground line and the cotyledonary node. It was hoped that by using large populations representative samples would be obtained.

The hypocotyls were collected on November 5, 7, 11, and 14; that is, 20, 22, 26, and 29 days after inoculation. Each specimen was cut to include the portion between the ground line and the cotyledonary node. If adventitious roots were present, these were included in the sample. Control and inoculated hypocotyls were weighed and then frozen by placing them in pyrex tubes immersed in a mixture of cellosolve and dry ice. After the material was frozen solid it was cut into smaller

pieces while still frozen, placed in chilled pyrex flasks, and dried in the vacuum (16). After the material was dry it was ground in a Wiley micro-mill to pass 20, 40, and 60 mesh, thus insuring thorough mixing of the sample. The powder was weighed and then stored over P_2O_5 in vacuum desiccators. Dry weights were determined by oven drying at 100° C. over night. They are given in table 1.

For each sample 100 plants were collected, except on the last day when only 43 each of noninoculated and of inoculated plants were available. Table 1 shows that while both inoculated and noninoculated hypocotyls were growing during the period of collection, the inoculated were enlarging more rapidly than the noninoculated, and that on the last two days the former on the average weighed al-

TABLE 1

NUMBER, WET WEIGHT, AND PERCENTAGE DRY MATTER OF NONINOCULATED AND INOCULATED HYPOCOTYLS OF TOMATO

Date of collection	Noninoculated			Inoculated		
	Number	Wet weight (gm.)	Dry weight (oven dried) (%)	Number	Wet weight (gm.)	Dry weight (oven dried) (%)
11/5/40	100	44	9.09	100	70.0	9.79
11/7/40	100	40	9.39	100	76.5	9.31
11/11/40	100	47	9.43	100	95.0	9.40
11/14/40	43	22.5	10.09	43	43.0	10.02

most twice as much as the latter. Practically all the growth at this time was diameter increase.

Figure 1 shows specimens exhibiting extreme difference as to length, diameter, root development, and gall diameter at time of collection. *A* and *B* are noninoculated, *C*–*E* are inoculated, and all are of the same age and cultural conditions. The cotyledonary node is indicated by a line drawn with india ink. In the main the inoculated hypocotyls differed from the noninoculated by being slightly shorter and slightly thicker, and by bearing more adventitious roots in addition to large tumors. These tumors were less green than the hypocotyl.

Since in preparation of the samples each hypocotyl was cut at the cotyledonary node, the coleoptile assays apply to whole noninoculated (plus such roots as they bore) as compared with whole inoculated hypocotyls (plus such roots as the hypocotyl and gall bore).

It was assumed that this is a better basis for sampling than use, on the one hand, of gall tissue alone and, on the other, of equal weights of contiguous tissue of the same hypocotyl, or of comparable tissue from a noninoculated hypocotyl, because it is not possible to define a sharp boundary of gall and non-gall

tissues. The abnormal increase in number of adventitious roots in the inoculated hypocotyl is clear indication that the auxin disturbance is not limited to the immediate site of the gall. The effect of inoculation with the parasite upon epinasty, adventitious root development, abscission of petiole, bud initiation, and cambial activity in regions remote from the site of inoculation and tumor development (**14, 17, 18**) indicates that if gall development is characterized by auxin disturbance (dysauxiny), this is not limited to the site of tumor production.

Since tests (**16**) with nodules and legume roots had indicated that dry ether extracts of thoroughly dry lyophilized material are auxinically inactive, and that

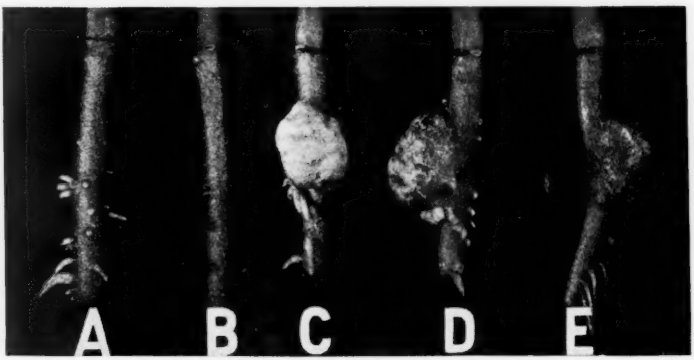

FIG. 1.—Tomato hypocotyls of same age (57 days) and cultural conditions. *A*, *B*, noninoculated; *C–E*, inoculated with *Phytomonas tumefaciens*. Photographed 29 days after inoculation. Natural size.

neither alcohol nor water alone is as effective an extractant as wet ether, this solvent was generally used. Similar results were reported by THIMANN and SKOOG (**31**) for oven-dried material. All ether used was distilled over wet $FeSO_4$ and CaO to eliminate peroxides. For experiments requiring the use of dry ether, the peroxide-free ether was first treated with $CaCl_2$ and then distilled and stored over metallic sodium. Other extractants and procedures are noted in the section on experiments and results.

After extraction, decanting, and filtration, the extracts generally were evaporated to dryness in a water bath at 50°–60° C. and taken up in 1 cc. of 1.5 per cent agar, from which dilution series were prepared. The agar was cast into a block, volume 120 mm.^{3}, which was then cut into twelve blocks of equal volume, 10 mm.^{3} Auxin assays were made by the standard *Avena* test (**33**) using an IDP time of 2–2.5 hours and a photographing time of 100 minutes. Each day the plants were calibrated with standard agar blocks containing indoleacetic acid, 0.015 and 0.030 mg. per liter of agar. The results of each test are given in arbitrary units, the unit being taken as that amount of auxin in 1 cc. of agar which gives the concen-

tration that produces 1° curvature of the *Avena* coleoptile under standard conditions. All units are calculated on the basis of 1 gm. of dry weight material (10 gm. wet weight) in 1 cc. of agar, and an average sensitivity of *Avena* coleoptile of 16° curvature for 30 γ indoleactic acid per liter of agar. The data are based on tests with 10,484 *Avena* plants.

Experimental results

Extractants and extraction procedures

Dry ether as extractant and solvent.—Three samples, each 0.1 gm. dry weight of gall material (third collection), were treated with 50 cc. of dry peroxide-free ether by standing in the ether for 3 days in the icebox at 2° C. Each sample was decanted and filtered. The filtrate of one sample was evaporated to dryness, taken up in agar, and assayed. No activity was obtained. The powder was re-extracted for 3 days with 50 cc. of dry ether plus 1 cc. H_2O by standing in the ice-box. The filtrate was evaporated to dryness, taken up in agar, and assayed. An activity of 210 units was obtained.

The dry ether filtrate of the second sample was evaporated and assayed. The extract was inactive. The powder was re-extracted and the filtrate evaporated as in the first sample. The residue was dried in an Abderhalden vacuum drier and then treated with dry ether which was filtered and evaporated. This residue was taken up in agar and assayed, to determine whether tomato auxin is soluble in dry ether. The activity was 105 units. To the dry ether filtrate of the third sample, 3 cc. of water was added. The water-ether filtrate was evaporated and the residue taken up in agar and assayed to determine whether a dry ether extract will become auxinically active upon addition of water. No activity was obtained.

The results of these tests with tomato tissue indicate (*a*) that after contact with thoroughly dry tissues, dry ether does not contain auxin; (*b*) that after contact with such material, wet ether does contain auxin; (*c*) that auxin from the hypocotyl is soluble in dry ether; (*d*) that presence of active auxin in the wet ether is not due to activation by water of a preauxin soluble in dry ether; but (*e*) that water itself in some way plays a role in rendering either active or potential auxin, or both, available for extraction by ether. These findings are in harmony with those previously reported for *Lemna* and other plants and for legume nodules and roots (**31, 16**). The results raise the question as to what happens to free auxin, that is, to the auxin present in fresh tomato tissue when these tissues are frozen and dried in vacuum. Possibly it is destroyed or it may be adsorbed and rendered nondiffusible by the freezing or the drying. At present we have no answer to these questions. Thimann and Skoog (**31**) report that drying may either destroy or fix auxin, or both, depending upon the auxin source.

Water as extractant and solvent.—Since the experiments with dry and wet

ether indicated that water plays a role in extraction of auxins from tomato tissues, a few preliminary experiments were made to test the effectiveness of water as extractant and the role of the time factor. In the first experiment 0.1 gm. of dry powder of the gall material (third collection) was treated with 2 cc. of water for 24 hours. Equal volumes of the filtrate and of 3 per cent agar were mixed and assayed. An activity of 188 units was obtained.

In a second set of tests, 25 cc. of water was added to 0.1 gm. of powder of the inoculated hypocotyl (third collection) and set in the icebox at 2° C. for 3 hours. After filtration, the filtrate was frozen and evaporated to dryness in the vacuum system. The residue was taken up in wet ether, evaporated to dryness, and then taken up in 1 cc. of agar and assayed. An activity of 160 units was obtained. To a second 0.1-gm. sample of powder, 50 cc. of water was added and the sample set in the icebox at 2° C. for 6 days. The filtrate was frozen, dried in vacuum, and assayed. An activity of 720 units was obtained.

In this connection an experiment was run to determine whether ether effectively removes tomato auxin from water. To this end a 0.1-gm. sample of the gall material (third collection) was shaken for 3 hours at room temperature with 25 cc. of water and then shaken four times with four volumes of ether. The ether residue was filtered, evaporated, and the residue assayed. An activity of 216 units was obtained compared with 208 units for an identical sample shaken at the same temperature with 50 cc. of wet ether.

These results for tomato are in line with those of AVERY, CREIGHTON, and SHALUCHA (**2**), who report that water is an effective extractant of auxins of corn endosperm, and the report of THIMANN and SKOOG (**31**) that ether effectively removes *Lemna* auxin from water.

WET ETHER AS EXTRACTANT.—Wet ether has been most generally used as extractant of auxin and found best for *Lemna* (**31**) and for legume nodules and roots (**16**). To test its efficacy as extractant and the role of the time factor, 0.1-gm. samples of gall material (third collection) were extracted with 500 volumes of wet ether freshly distilled over wet $FeSO_4$. In two instances, parallel extractions were made by placing one lot of the material on a shaker by the side of the material extracting by standing, to test the effect of constant agitation and hence presumably of better contact between extractant and powder used in these tests.

The results (table 2) show that relatively small volumes of wet ether are fully as effective extractants as equal volumes of water alone. The results also show that a relatively large amount of auxin is extracted in a short time, and that the amount increases slowly but significantly with lengthening time periods. The shaken samples showed an increase for the 24-hour but not for the 3-hour lot. This small increase and the other observations just made indicate that the slow liberation of auxin is not due primarily to the state of division of the material but

to a slow process which goes on in the presence of water. This is in harmony with the findings of THIMANN and SKOOG (**31**), who report that for *Lemna* an equilibrium probably exists between the water-plant material and the water-ether phases.

EXTRACTION BY SOXHLETIZATION

Two series of Soxhlet extractions with wet ether were run to test the effectiveness of this method. A preliminary test was made to determine whether the auxin fraction extractable from inoculated hypocotyls is affected by the temperature of boiling ether. To this end 100 cc. of wet ether was added to 0.2 gm. dry weight of the inoculated hypocotyl (third collection) and allowed to stand $3\frac{1}{2}$ days at 2° C. in the icebox. The filtrate was divided into two equal portions. The first

TABLE 2

AUXINIC ACTIVITY (IN UNITS) OF WET ETHER EXTRACTS OF 0.1-GM. DRY WEIGHT SAMPLES OF INOCULATED HYPOCOTYL

TIME (HOURS)	ACTIVITY	
	WITHOUT SHAKING	SHAKEN
3	208	208
24	260	340
72	616	.....
84	560	.....
144 (*a*)	600	.....
144 (*b*)	800	.....

was evaporated and assayed and the second was boiled with ether in a reflux system for 24 hours and then evaporated and assayed. An activity of 560 units was obtained for each sample, indicating that the auxin fraction from tomato—like that of legumes (**16**)—is stable at the temperature of boiling ether. THIMANN and SKOOG (**31**) concluded that the auxin is destroyed by continued boiling in ether.

Another preliminary test was made—using dry ether—to determine whether the results obtained from standing in dry ether would apply. Tests were made with gall material from each of the four collections, and in each case the results were negative. Each sample, 0.1 gm. dry weight, was Soxhletized for 3 hours with dry peroxide-free ether.

Next, two sets of tests were made with wet ether on 0.1-gm. samples of gall material (third collection). The ether was not of the same lot for both sets and consequently probably did not contain the same amount of water. In the first set three samples were Soxhletized for 6, 12, and 24 hours, respectively, and in the

second set for 3, 6, and 12 hours, respectively; and after the first treatment, the 6-hour sample was extracted for another 6 hours with a new lot of ether and the 12-hour sample for another 12 hours with a new lot of ether.

The results are given in table 3, and indicate that Soxhletization with wet ether is a more efficient method of extraction of tomato material than standing in wet ether or in water, two 12-hour extractions yielding almost as much auxin as 144 hours of standing in water or in wet ether. THIMANN and SKOOG (**31**) report that the yields of auxin from *Lemna* by Soxhletization with ether were lower than those obtained by standing in wet ether in the cold. VAN OVERBEEK (**32**) reports

TABLE 3

AUXINIC ACTIVITY (IN UNITS) OF WET ETHER EXTRACTS OBTAINED FROM 0.1 GM. OF INOCULATED HYPOCOTYL BY SOXHLETIZATION

TIME (HOURS)	ACTIVITY	
	FIRST EXTRACTION	SECOND EXTRACTION
1st series		
6	352	.....
12	340	.....
24	408	.....
2d series		
3	168	.....
6	520	0
12	624	40

that 24-hour Soxhletization of *Avena* coleoptile removes all auxin, including the active and the potential, which latter he considers a measure of the precursor present.

The yields for identical periods of extraction by Soxhletization and by standing in water or in wet ether varied considerably from test to test. This may be due to several factors, such as different pH, alone or together, with different amounts of water in the different ether lots or unequal initial and hence subsequent wetting of the lyophile powder.

COMPARATIVE ASSAYS OF AUXIN CONTENT OF NONINOCULATED AND INOCULATED HYPOCOTYLS

ASSAYS OF FOUR PAIRS OF SAMPLES COLLECTED 20, 22, 26, AND 29 DAYS AFTER INOCULATION.—For these assays 2-gm. samples (dry weight) were used. The materials collected 14, 20, 26, and 29 days after inoculation were tested in pairs, each pair consisting of noninoculated and inoculated hypocotyls for one date of collection. Freshly prepared peroxide-free ether (1000 cc.) was added to each sample

and allowed to stand in the icebox at 2° C. for 7 days. The extractant was decanted and filtered; the filtrate was evaporated to dryness, taken up in agar, and assayed. The results are given in table 4.

For each pair tested the gall extracts gave a markedly higher assay than the extracts of the noninoculated hypocotyl, the difference being least for the pair of the third collection. The differences are large enough to be significant, the ratios ranging from 1:5.4 to 1:15.

AUXIN ASSAY OF SUCCESSIVE EXTRACTIONS WITH WET ETHER.—There are many observations to the effect that a single extraction does not remove all auxin from

TABLE 4

AUXINIC ACTIVITY (IN UNITS) OF WET ETHER EXTRACTS OF NONINOCULATED AND INOCULATED HYPOCOTYLS BY STANDING IN REFRIGERATOR IN DARK AT 2° C. FOR 7 DAYS; 2.0 GM. (DRY WEIGHT) OF MATERIAL AND 1000 CC. OF ETHER PER SAMPLE

DATE OF COLLECTION (1940)	AUXINIC ACTIVITY	
	NON-INOCULATED	INOCULATED
11/5, 20 days after inoculation	38	215
11/7, 22 days after inoculation	32	480
11/11, 26 days after inoculation	80	440
11/14, 29 days after inoculation	40	430

plant material (**31, 16**). The results recorded in the first section of this paper indicated that this applies also to the crown-gall material. To determine whether this applies to the noninoculated hypocotyl as well, and what amounts could be obtained in successive extractions, inoculated and noninoculated samples of the third collection were subjected to repeated extractions, beginning December 5, 1940. The last test recorded was made on July 24, 1941, after 209 days of extraction. Since the extracts were active on the last date of test, the extraction is being continued.

In the tests, 2 gm. each (dry weight) of the noninoculated and of the inoculated hypocotyl of the third collection (November 11, 1940) were covered each with 1000 cc. of freshly distilled peroxide-free ether and allowed to stand in the icebox at 2° C. for 7 days, with occasional shaking. The extractant was decanted, filtered, evaporated, and assayed (December 18). After the first assay the powders were dried and stored in a vacuum desiccator over P_2O_5 until January 3. They were then extracted for 24 hours each with 500 cc. ether. The extracts were decanted and tested on January 4 and the materials covered again with ether. After 7 days another assay was made (January 11). The procedure was repeated,

the periods of extraction varying from 4 to 42 days and the amounts of ether ranging from 250 to 500 cc. The results are given in table 5.

Throughout the 209 days of extraction, and on each of the seventeen successive extractions, the gall-bearing hypocotyl gave a higher auxin assay than the noninoculated hypocotyls of the same age and cultural conditions. The amount of difference varied from extraction to extraction, the greatest contrast being noted

TABLE 5

AUXINIC ACTIVITY (IN UNITS) OF EXTRACTS OF NONINOCULATED AND INOCULATED HYPOCOTYLS THROUGH SUCCESSIVE EXTRACTIONS WITH WET ETHER BY STANDING IN REFRIGERATOR IN DARK AT 2° C.; 2.0 GM. (DRY WEIGHT) USED FOR EACH SAMPLE; 1000 CC. ETHER USED FOR FIRST EXTRACTION, THEN 500 OR 250 CC.

TREATMENT AND DATES OF TEST	AUXINIC ACTIVITY	
	NON-INOCULATED	INOCULATED
7 days in ether, 12/18/40	80	440
Redried powder kept in desiccator then in ether 24 hours, 1/4/41	0	74
7 days in ether, 1/11/41	30	450
4 days in ether, 1/15/41	26	120
7 days in ether, 1/22/41	30	80
7 days in ether, 1/29/41	40	100
7 days in ether, 2/5/41	12	100
7 days in ether, 2/12/41	26	76
6 days in ether, 2/18/41	14	32
8 days in ether, 2/26/41	14	61
7 days in ether, 3/5/41	16	48
7 days in ether, 3/12/41	10	40
15 days in ether, 3/28/41	19	72
21 days in ether, 4/17/41	16	44
22 days in ether, 5/9/41	11	38
42 days in ether, 6/20/41*	35	91
Total units	379	1866

* In a test on 7/24/41 the noninoculated assayed 20 units and the inoculated 62 units.

after the third extraction. During the first three extractions more than half the total obtained was extracted from the gall-bearing tissues, while less than one-third was extracted from the noninoculated hypocotyl. In this respect the gall material behaved like the legume nodule material (**16, 31**) and the noninoculated hypocotyl like roots of legumes, *Lemna*, and *Avena*. The two samples also differed as to rate and amount of swelling in water, the noninoculated swelling more rapidly and completely; as to amount of chlorophyll and other pigments liberated, the noninoculated yielding more chlorophyll; and as to extent and rate of browning, the noninoculated browning only slowly and slightly while the inoculated browned

rapidly and extensively, indicating a higher incidence of oxidizing enzymes for the latter, as reported by NAGY and RIKER (**25**). The second assay indicates that auxin liberation from the noninoculated hypocotyl is affected more deleteriously by drying than that of the inoculated.

Both samples are alike in that 209 days of extraction did not suffice to yield an auxin-free extract. In the light of GUSTAFSON'S report (**7**) that tomato seedlings yielded auxin after more than a year's extraction, these samples are likely to yield auxins for a long time. Unfortunately this means that the basis for developing a simple quantitative extraction method for this material is not yet at hand, because the total auxin content (active plus potential) cannot be determined.

SHORT EXTRACTIONS WITH WET ETHER.—In these tests 0.2 gm. (dry weight) of the noninoculated and 0.1 gm. (dry weight) of the inoculated hypocotyl were extracted with wet ether by standing for 5, 20, and 60 minutes, respectively, at 2° C. in the refrigerator. The extractants were decanted, filtered, and assayed. The noninoculated materials gave yields of 0 units in 5 and 20 minutes, and of 10 units in 60 minutes; the inoculated gave yields of 98 units in 5 minutes, 132 units in 20 minutes, and 145 units in 60 minutes.

WATER AS EXTRACTANT.—In this test 0.1 gm. each (dry weight) of the noninoculated and inoculated hypocotyl (third collection) were treated each with 2cc. of water for 24 hours by standing in the icebox at 2° C. Equal volumes of the filtered extractant were mixed with equal volumes of 3 per cent agar and assayed. The noninoculated hypocotyl yielded 38 units; the inoculated, 188.

YIELDS OBTAINED BY SOXHLETIZATION.—In the first experiment materials of the third collection were used, 0.2 gm. (dry weight) each being extracted for 6 hours in micro-Soxhlets and another pair for 12 hours. After extraction, the powder was placed in wet ether (50 cc.) for 7 days at 2° C. The extracts of the two series were made on different days with different lots of ether. The results are given in table 6. Comparative tests also were made with noninoculated and inoculated hypocotyls of the first and second collections. In these tests 0.5-gm. samples were extracted for 3 hours. After this the powders were extracted with wet ether for 24 hours at 2° C. in the icebox. The results are given in table 7. A comparative test was made for materials of the third collection by Soxhletizing it for 3 hours with dry ether. Neither inoculated nor noninoculated hypocotyls yielded an active extract.

CONTINUOUS ETHER EXTRACTION.—For this test, which supplements the Soxhlet extractions, 0.1 gm. each (dry weight) of the noninoculated and inoculated hypocotyl (third collection) were covered with 15 cc. of water in a continuous extractor and extracted for 1 hour each with ether. The extractants were filtered, evaporated, and assayed. The former yielded no measurable amount; the latter, 90 units.

These results support those for wet ether extracts to the effect that the extracts

of the inoculated hypocotyl are more active auxinically than are those of the noninoculated. Soxhletization proved the best short method for extraction of the noninoculated hypocotyl just as it has for the inoculated.

These observations may mean that the rate of liberation for auxin from the precursor (or bound form) is more rapid in case of the gall material, or that there

TABLE 6

AUXINIC ACTIVITY (IN UNITS) OF EXTRACTS FOLLOWING SOXHLETIZATION AND SUBSEQUENT EXTRACTION WITH WET ETHER

TREATMENT	NON-INOCULATED	INOCULATED
Soxhletized for 6 hours	38	528
Resoxhletized for 12 hours	33	220
Re-extracted by standing 7 days in wet ether	18	509
Total	89	1257

TABLE 7

AUXINIC ACTIVITY (IN UNITS) OF ETHER EXTRACTS AFTER SOXHLETIZATION AND SUBSEQUENT EXTRACTION WITH 50 CC. WET ETHER BY STANDING IN REFRIGERATOR AT 2° C. FOR 24 HOURS; 0.5 GM. (DRY WEIGHT) FOR EACH SAMPLE

DATE OF COLLECTION	TREATMENT	NON-INOCULATED	INOCULATED
7/5/41	3-hour Soxhletization	0	18
	Re-extracted with wet ether for 24 hours by standing	8	160
Total		8	178
7/7/41	3-hour Soxhletization	0	24
	Re-extracted with wet ether for 24 hours by standing	28	240
Total		28	264

is more auxin immediately available in the gall, or both. They may even mean that the auxins of the noninoculated material differ in solubility, equilibrium relations, methods of liberation, or in their initial sources (precursors) from the auxins of the inoculated materials.

EFFECT OF BOILING ON SUBSEQUENT AUXIN LIBERATION.—The preceding experiments show that lyophilized tomato materials do not yield auxin to dry ether,

even though the auxin is soluble in dry ether, but that wet ether extracts are auxinically active. This, together with the fact that fresh tomato material diffuses auxin into agar and yields auxin when extracted with wet ether, alcohol, or chloroform, means that freezing, or drying in vacuum, or both, destroy the active (diffusible and extractable) auxin present in fresh material, or that they fix or bind it to the treated tissues, as was reported for *Lemna* (**31**) and legume nodules and roots (**16**). It has been suggested by these investigators that water plays a role in liberating the fixed auxin by hydration.

The observation that many plant tissues over long periods liberate auxin in excess of the diffusible and initially extractable fraction suggests that water also plays a hydrolytic role in the process of enzymatic liberation of potential auxins from precursors. SKOOG and THIMANN (**28**) report that addition of proteolytic enzymes to a water suspension of *Lemna* material greatly accelerated the rate of auxin liberation. This suggests that if the enzyme systems, or the precursor involved, or both, could be destroyed without loss of the free auxin present, a single extraction might show the amount initially present. THIMANN and SKOOG (**31**) performed such experiments by treating *Lemna* leaves with steam, or by boiling, before extraction and found that this procedure did not affect the initial yield but stopped prolonged liberation of auxin. Their results indicate that an enzyme system is involved and that continued liberation of auxins is dependent upon bound auxin.

To determine whether these findings apply to noninoculated and inoculated hypocotyls of the tomato, some of our material was put to test by heating prior to extraction with wet ether. A preliminary test was made to determine the effect of boiling water upon the ether extracts of noninoculated and inoculated hypocotyls. A 0.5-gm. sample (dry weight) of the noninoculated (fourth collection) and a 0.1-gm. sample (dry weight) of the inoculated hypocotyl (third collection) were extracted by standing in wet ether for 3 days. Each extract was filtered and divided into two equal parts, one of which was evaporated and assayed while the other was evaporated, taken up in 0.5 cc. of water, boiled for 5 minutes, diluted with an equal volume of 3 per cent agar, and then assayed. The noninoculated material, unboiled, gave an activity of 25 units; the boiled, of 26 units; the inoculated material, unboiled, gave an activity of 581 units, and boiled, of 582 units—indicating that tomato auxin is not destroyed by boiling in water.

For the test itself a 0.5-gm. sample (dry weight) of the noninoculated and a 0.1-gm. sample (dry weight) of the inoculated hypocotyl (third collection) were placed in Soxhlet thimbles and immersed in 5 cc. of boiling water for 5 minutes, with stirring to prevent excessive clumping. The water extracts were shaken four times with four volumes of ether and the ether extracts filtered, evaporated, and assayed for auxins. The thimbles were dried in a stream of air and then placed in

the Soxhlet with wet ether for 24 hours. The extractant was filtered, evaporated, and assayed. The material then was given another extraction in the Soxhlet for 15 hours with wet ether, and a third one by standing in wet ether for 4 days at 2° C. Next each sample was incubated at 37.5° C. for 24 hours with 1 mg. of crystalline chymotrypsin per 0.1 gm. of material in water and a trace of toluene, adjusted to pH 9 with M/2 Na_2CO_3 (**28**). After filtration the pH was adjusted to 4 and the filtrate was extracted by shaking with four volumes of ether. The ether extract was evaporated and tested. The results are given in table 8.

The auxin recovered by the first Soxhletization is taken to be free auxin present at the moment the tissues were frozen. The inoculated hypocotyl contains more of this than the noninoculated. The absence of auxin in the extract of the second

TABLE 8

AUXINIC ACTIVITY (IN UNITS) OF NONINOCULATED, 0.5 GM., AND INOCULATED HYPOCOTYLS, 0.1 GM. (DRY WEIGHT), AFTER TREATMENT WITH BOILING WATER, WITH CHYMOTRYPSIN, AND WET ETHER

Boiling water extraction 5 minutes		Then 24-hour Soxhletization with ether		Then 15-hour Soxhletization with ether		Then 7-day extraction standing in ether		Then 24-hour treatment with chymotrypsin and extraction with ether	
Noninoculated	Inoculated	Noninoculated	Inoculated	Noninoculated	Inoculated	Noninoculated	Inoculated	Noninoculated	Inoculated
0	0	10	70	0	0	0	0	10	20

Soxhletization and in the extract by standing in wet ether for 4 days is taken to signify that boiling destroyed either the agent by which, or the substance from which, potential auxin is liberated, or both. The presence of auxin in the extract following treatment with chymotrypsin is interpreted as meaning that boiling destroyed the enzyme which liberated auxin rather than the precursor, and that a proteolytic enzyme can liberate auxins from the cell constituents of higher plants.

The low yields of auxin, 10 and 20 units out of a possible 399 and 1858 units, respectively, for the noninoculated and inoculated hypocotyls, following digestion with chymotrypsin, indicates that (*a*) either the enzyme or the conditions during digestion were not favorable for maximum hydrolysis, or (*b*) most of the precursor had been altered by boiling so that auxin no longer could be liberated, or (*c*) proteins are not the only precursors of auxins. The toluene used to inhibit auxin formation by bacteria and fungi probably is not responsible for the low yields. In tests with agar containing extracts of the noninoculated hypocotyl with and without toluene, the former assayed 11.6 and the latter 11.4 units, indicating that the small amount of toluene used had affected neither the sensitivity of the coleoptiles nor the activity of the extract.

The fact that the amount of free auxin recovered from the inoculated hypocotyl is 70 units, while 98, 132, 145, and 720 units are obtained by extraction for 5, 20, 60 minutes, and 7 days, respectively, indicates that the gall at any moment contains a relatively large amount of free auxin which is liberated rapidly in extraction, but that the formation of auxin from its precursor proceeds slowly and at a decreasing rate.

The noninoculated hypocotyl behaves essentially like the inoculated, but differs quantitatively with a free auxin content of 20 units, no measurable liberation of auxin in 5 and 20 minutes, and a yield of but 10 units in 60 minutes and of 80 units in 7 days.

Discussion

The data show that extracts of the gall-bearing hypocotyls give higher free and potential auxin assays than the extracts of the normal and healthy hypocotyls. This holds for all pairs of samples tested and for all extractants and methods of extraction tried.

These findings are at variance with those reported by RIKER, BERCH, and DUGGAR (**27**). A comparative critique of the diametrically opposed data must await a further report by these investigators. The differences may be due to sampling methods, to nature of the samples, to methods of extraction, or to combinations of these. Extensive collections have been made to determine whether the findings reported here hold for other materials collected throughout the growing period of the tomato plant, and whether the gall material differs auxinically from contiguous tissue of the gall-bearing hypocotyl.

No diffusion tests have been made in the course of this investigation. Earlier ones conducted in this laboratory with old galls were negative, and LOCKE, RIKER, and DUGGAR (**17, 18**) report no success with diffusion tests on old galls. The basis for this failure possibly may lie in the same factor responsible for the observation by THIMANN and SKOOG (**31**) that while tomato callus grown in tissue culture yields auxin upon extraction, it does not liberate any by diffusion. They attribute this to disturbance in the polarity relations of the tumor tissues.

The fact that the gall tissues yield more auxin than the normal is in harmony with the findings of LINK, EGGERS, and MOULTON (**16**) that legume nodules yield more auxin than legume roots, and of MOULTON (**24**) that the smut galls of corn yield more auxins than comparable healthy tissues. It gives support to the hypothesis (**12, 14, 33**) that gall development, and in particular crown-gall development, is characterized by disturbed relations of growth substances (dysauxony), and that disturbed auxin relations (dysauxiny) induced directly or indirectly by wounding and by the parasite, through effects upon the host's metabolism, are part of the causal complex of crown-gall development. This hypothesis—if experimentally substantiated—gives support to the generally accepted corollary hypothesis that auxins play some role in the causal complex of normal and of

healthy growth. Any finding that abnormal auxin relations are not causally related to abnormal growth relations would seriously—but perhaps not fatally—weaken the auxin hypothesis for normal growth.

No conclusion can yet be drawn as to whether the hyperauxiny of the diseased hypocotyl is due (*a*) solely to auxins formed by the parasite; (*b*) solely to increased local auxin development by the host (with or without auxin increase in distal parts) and increased auxin transport to the site of gall production; or (*c*) to combinations of these. The senior writer has inclined from the beginning (**14, 15**) to the hypothesis that in addition to auxones, including auxins furnished by the parasite, the host cells—local and distant—also contribute to the hyperauxony of the affected organ. LEONIAN advanced the same view (**11**). Evidence for this interpretation is supplied by the observations of LOCKE, RIKER, and DUGGAR (**17, 18**) on the behavior of plant parts remote from the site of gall production.

The studies of NAGY and RIKER (**25**) on the chemical and physiological constitution of crown-gall tissue versus contiguous tissues corroborate and expand an earlier summary (**26**) to the effect that the chemical constitution of crown-gall tissue differs profoundly from that of healthy tissue, that the metabolism of the gall is at a higher level than that of contiguous tissues, and that the gall tissue approximates the chemical constitution and behavior of growing points.

The gall tissue is richer than the contiguous tissues in ash, ether extracts, total nitrogen, and simple forms of nitrogen. The polypeptide and protein fraction is two to four times greater in the gall than in the normal tissues. Glutathione and ascorbic acid are more abundant in the gall tissue; and the activity of oxidizing enzymes, catalase, peroxidase, and oxidase is higher. The tyrosinase activity of the gall tissue is 20–200 per cent greater than in the normal tissues. Our observations on the behavior of the noninoculated and inoculated tissues during extraction are in line with the reports regarding oxidizing enzymes. The finding that the polypeptide content of the galls is greater than that of the normal tissue is significant in that it is possible that amino acids and proteins are the precursors of auxins, and in that bacteria and fungi readily convert tryptophane to indoleacetic acid (**33**).

Biochemical studies of tumors induced by treatment with idoleacetic acid and similar auxins and with other auxones show that these pathic growths have much in common with tumors which are incited by parasites and are properly designated galls. Following the work of ALEXANDER (**1**) and COOPER (**6**) with indoleacetic acid and of STUART and MARCH (**30**) with indolebutyric acid, which showed that the local application of these substances not only mobilizes substances in distant parts of the plant but also leads to their transport and accumulation in the treated organ, and of BORTHWICK, HAMNER, and PARKER (**4**) which showed that proteins increase and nitrates decrease in the zone treated with indoleacetic acid and that there are changes in starch content, a series of researches

has established these findings in principle and in greater detail. MITCHELL and MARTIN (**20**) showed that in etiolated beans a local and systemic disturbance of digestion and translocation results upon application of indoleacetic acid. STUART (**29**) showed that there is abnormal movement of nitrogen and of carbohydrates to the site of treatment from distant parts following application of indolaecetic acid, and that there is increase in dry weight and of water, sugar, nitrogen, and calcium pectate in the treated zone. MITCHELL and HAMNER (**21**) and MITCHELL and STUART (**22**) also showed that the effects for indoleacetic acid are closely related to the concentration of the material applied. MITCHELL and WHITEHEAD (**23**) report that spraying of plants with auxins, including indoleacetic acid, is followed by increase in starch digestion. Similar results have been found by KRAUS and MITCHELL (**10**) and by MITCHELL (**19**) for substances such as alpha naphthalene acetamide and naphthaleneacetic acid, which are not known to occur in plants. These results and those of BEAL (**3**) with excised tissues indicate that local auxin and other auxone disturbances not merely mobilize materials in distant parts but also set up gradients or fields in movement and accumulation of materials which in some way are part of the causal complex of excessive local growth and development. Auxin apparently behaves not only as a mobilizer, but even more definitely as an incitant and focalizer of transport in the new gradients or fields which it establishes in the affected plant.

It has been reported (**13, 14**) that the crude auxin extract of *P. tumefaciens*, which BROWN and GARDNER (**5**) also had found to be active, gave the color test with ferric chloride, which is produced by indoleacetic acid. What auxin or auxins actually enter into the hyperauxiny of crown gall of tomato remains to be determined. HAAGEN-SMIT, LEECH, and BERGEN (**8**) have reported that an auxin extract from corn seed contains mainly indoleacetic acid and a small amount of pseudo-auxin a. This first report of the isolation of crystalline indoleacetic acid from green plant tissues weakens those criticisms in discussions of the crown-gall and legume nodule problems (**9, 12, 14, 18**) based on the fact that indoleacetic acid had not been isolated from green plants.

The questions whether the coleoptile tests measure the net effect of several auxins or even of auxins and of auxin inhibitors; whether the coleoptile test detects all growth substances (auxones); whether the auxin in crown gall is indoleacetic acid or indoleacetic acid plus other auxins; and lack of knowledge as to how these effects are brought about in the cells—these questions do not affect the fact that the coleoptile test reveals a greater content of growth-promoting substances in extracts of tomato crown gall than in extracts of the noninoculated hypocotyl. The finding at least shows that in one more respect gall tissue differs chemically and dynamically from normal healthy tissue of the same plant. These results, based on inoculation of an organ by a parasite but without decapitation, supplement and augment those auxin studies which utilize another line of pathological

evidence, that is, chirurgic mutilations in the form of decapitations of plants plus chemical infection in the form of applications of plant extracts and synthetic chemicals. These pathological techniques and data have to date furnished the main experimental basis for the physiological theory of growth substances.

Before a more successful approach can be made to the main problems still outstanding, among them estimation, isolation, and identification of the auxones involved in crown gall as compared with healthy tissues, and the role of these substances in healthy and in pathic growth, it is necessary to devise methods of extraction which are more quantitative than those available at present. None of the methods tried in these tests is satisfactory for determination of total auxins of tomato. Soxhletization with wet ether for 24 hours of material previously boiled for 5 minutes in water apparently suffices for removal of all free auxin. We have some evidence that a high pH of the extractant and of the tomato materials favors extraction by water and ether, and that digestion with proteolytic enzymes of materials freed from free auxins may provide a method for determination of the potential auxin.

Summary

1. The extraction of auxins from hypocotyls of tomato, noninoculated and inoculated with the gall-inciting schizomycete *Phytomonas tumefaciens*, has been studied.

2. Frozen vacuum-dried (lyophilized) tomato material does not yield auxins to dry ether. The immediately available or free auxin seems fixed until water liberates it.

3. Wet ether gave the best results as extractant, but even after seventeen successive extractions during 209 days, the process of auxin liberation had not reached completion.

4. Material boiled before extraction yields all its free auxin in one extraction by Soxhletization with wet ether for 24 hours, whereas non-boiled material continues to yield auxin. By combination of these methods the free auxin content may be distinguished from the potential or bound auxin. The latter may be a measure of auxin precursors, and is obtainable in part by digestion with chymotrypsin in the presence of toluene, from material freed from free auxin.

5. The inoculated hypocotyl yields both more free and more potential auxin than the noninoculated.

6. The significance of the finding that the growth disturbance known as crown gall is associated with disturbance in auxin relations (dysauxiny) is discussed with reference to the hypothesis that auxins play roles in normal and in healthy growth of plants and to the causal complex of gall development.

UNIVERSITY OF CHICAGO

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RESPONSES OF TWO SPECIES OF TOMATOES AND THE F_1 GENERATION TO SODIUM SULPHATE IN THE NUTRIENT MEDIUM[1]

C. B. LYON[2]

Introduction

It has been recognized for some time that varieties of a given species of plant differ in their response to various environments. Inbred strains of corn and their F_1 generations have been found to vary in growth responses when available soil moisture was altered (**13**), when grown on rich and poor soil types (**12**), and when various fertilizer treatments were used (**3**). SMITH (**20**), using inbred strains and single crosses of corn, found marked differences in growth in relation to the phosphorus and nitrogen content in nutrient solutions. LYNESS (**15**) used sand cultures and reported results essentially in agreement with those of SMITH. HARVEY (**9**) found a differential utilization of ammonium and nitrate nitrogen among inbred lines of tomato and corn with their hybrids, and BURKHOLDER and MCVEIGH (**2**) found that inbred lines of corn and their hybrids differed in their responses to nitrogen supply in sand culture. Definite physiological symptoms of tomato plants in relation to high concentrations of Na_2SO_4 have been reported by EATON (**6**) and HAYWARD and LONG (**11**), using a commercial strain of the Marglobe variety.

The experiment here reported was designed to test the effects of high concentrations of sodium sulphate on (*a*) two species of tomato which are as completely homozygous as it is practicable to obtain, and (*b*) the heterozygous F_1 generation. Records were made of fruit production, anatomy of the stem, and growth responses.

Material and methods

The tomato, *Lycopersicum esculentum* Mill., variety Johannisfeuer, and *L. pimpinellifolium* (Jusl.) Mill., the Red Currant variety, together with the F_1 generation, were used. It is an interspecific cross, and the two species differ in many plant characters. The parent strains had been inbred under controlled conditions of pollination for at least five generations, and inasmuch as LINDSTROM (**14**) has found only 1–3 per cent of natural crossing under field conditions, they were considered essentially homozygous. POWERS (**18**), POWERS and LYON (**19**), and LYON (**16**) have reported the inheritance of some of the quantitative characters.

Seeds of each of the three lines were planted in flats containing sand on May 18,

[1] Investigation conducted at U.S. Regional Salinity Laboratory, Riverside, California.

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1940, and germinated in the greenhouse.[3] Individual plants were transplanted on May 31 to 5-gallon glazed crocks filled with quartz sand containing 0.3 per cent of magnetite, which has been found (**4**) to provide sufficient iron for plant needs. The crocks were placed outdoors in large automatically operated nutrient culture tanks designed and described by EATON (**4, 5**). Eight plants were used in each tank, and the equipment provided 4800 liters of nutrient solution for each tank. This solution was circulated through the crocks for a period of 2 minutes during each hour from 6:00 A.M. to 6:00 P.M., with one additional irrigation at midnight. Each irrigation more than displaced the solution held in the crock. The plants were trained upright, and all axillary growth was pruned off twice weekly.

TABLE 1

COMPOSITION OF NUTRIENT SOLUTIONS

TREATMENT	P.P.M.		MILLIEQUIVALENTS PER LITER								
	B	Mn	Ca	Mg	Na	K	HCO_3	SO_4	Cl	NO_3	H_2PO_4
Tap water*	0.1	.....	1.8	0.6	1.6	0.2	2.9	0.6	0.6	0.1	.....
a) Base nutrient	1.0	0.2	6.2	4.6	1.6	3.4	2.9	4.6	3.0	8.1	0.6
b) Base nutrient+40 m.e. Na_2SO_4	1.0	0.2	6.2	4.6	41.6	3.4	2.9	44.6	3.0	8.1	0.6
c) Base nutrient+80 m.e. Na_2SO_4	1.0	0.2	6.2	4.6	81.6	3.4	2.9	84.6	3.0	8.1	0.6
d) Base nutrient+120 m.e. Na_2SO_4	1.0	0.2	6.2	4.6	121.6	3.4	2.9	124.6	3.0	8.1	0.6

* Analyses of tap water kindly supplied by Dr. A. D. AYERS of the Salinity Laboratory. Ionic concentrations remained practically constant.

The nutrient solution was composed of KNO_3, $MgSO_4$, KH_2PO_4, H_3BO_3, $MnCl_2$, Na_2SO_4, $CaCl_2$, and $Ca(NO_3)_2$. Four solutions were used, consisting of (*a*) control solution which constituted the base nutrient, (*b*) base nutrient plus 40 milliequivalents per liter of Na_2SO_4, (*c*) plus 80 milliequivalents per liter of Na_2SO_4, and (*d*) plus 120 milliequivalents per liter of Na_2SO_4. Analyzed commercial salts were dissolved in tap water as used at Riverside, California. Salts were added in addition to those present in the water to raise the total concentration of the various ions of the solution to the desired strength (table 1).

To avoid injury of the seedlings immediately after transplanting and to assure the ripening and maturation of twenty fruits per plant, all tanks were initially supplied with base nutrient solution, and Na_2SO_4 was added as required in five equal amounts until the desired concentrations were attained. The additions extended over a 12-day period, and were made on June 27, July 1, 3, 6, and 9, 1940, when the plants were 40–52 days old and a few of them were in bloom. Additional water was

[3] The seed was supplied through the courtesy of Dr. LEROY POWERS, Senior Geneticist, U.S. Horticultural Field Station, Cheyenne, Wyoming.

added to the reservoirs twice weekly to replace losses incurred through transpiration and evaporation. The pH of the nutrient solutions was checked daily with a Beckman pH meter and maintained at a pH of 5.5 by additions of HNO_3 when needed. The HNO_3 served the dual purpose of controlling the pH and maintaining nitrogen concentrations between 6 and 8 milliequivalents per liter (**7**).

After germination, the experiment was conducted outside, where a hygrothermograph placed near the plants recorded temperature and relative humidity. Pyroheliometer readings were obtained from the Citrus Experiment Station at Riverside, California, approximately 3 miles from the location of the experimental plots. The mean daily temperature from June 1 to September 15, 1940, was 74°.0 F., with a mean maximum of 92°.9 and a mean minimum of 55°.1 F. Highest temperatures were noted in July and August, with mean maximums of 96°.7 and 96°.0 F., respectively. During the experiment the mean relative humidity was 68.3 per cent at 8:00 A.M. and 38.0 per cent at 12:00 A.M. The average daily total of solar radiation during this period was 579.4 gram calories per square centimeter of horizontal surface, with the highest values between June 15 and July 15.

The design of the experiment was that of a randomized block (**8**) with four treatments and twelve tanks. Three tanks were used for each treatment, with eight plants per tank. Eight plants of a strain were grown in each treatment, and the three strains were replicated in each tank. The three tanks, as well as the three strains of tomatoes within each tank, were randomized by the use of TIPPETT'S randomization tables (**22**). The design provided for eight replications of each strain per treatment, with a total population of ninety-six plants. The data were reduced by means of the analysis of variance, and the t test (**21**) was used for determining whether particular differences were statistically significant. Odds as great as—or greater than—19.1 against the deviations, being due to the errors of random sampling, were accepted as statistically significant.

Experimentation and results

HEIGHT OF PLANTS

On August 13 the plants were 87 days old and had been growing on complete concentrations of their nutrient solutions for 35 days. At this time differences in plant heights were noted and recorded (table 2). There is a mean difference of 16.8 ± 6.57 cm. demonstrable between parental lines grown on the base nutrient solution, and the F_1 generation was 43.7 ± 6.89 cm. taller than the Johannisfeuer parent. Both of these differences are mathematically significant. The Johannisfeuer parent produced more growth in height of plant than the Red Currant parent, while the F_1 generation grew more rapidly than either parent. Although the magnitude of the differences between the strains, as well as the variability within them, are probably accentuated by pruning away all axillary growth, the relative

plant heights are in agreement with inherent differences demonstrated by POWERS (18).

The application of increasing concentrations of Na_2SO_4 does not affect the relative height of parental lines in comparable treatments or the phenomenon of heterosis shown in the F_1 generation. In a comparison of treatments *a* and *d*, significant mean differences of 17.9 ± 4.34 cm., 26.7 ± 6.63 cm., and 18.2 ± 5.87 cm. are demonstrable for Johannisfeuer, the F_1 generation, and Red Currant, respectively. Johannisfeuer plants grown in treatment *d* were 88.3 per cent as high as those in treatment *a*, while F_1 plants were 86.4 per cent as high, and Red Currant plants were 86.7 per cent as high on the basis of a similar comparison. For all

TABLE 2*

HEIGHT OF PLANTS IN CENTIMETERS

TREATMENT	STRAIN		
	JOHANNISFEUER	F_1 GENERATION	RED CURRANT
a) Base nutrient	153.3±3.42	197.0±5.98	136.5±5.61
b) Base nutrient+40 m.e. Na_2SO_4	152.0±2.52	191.6±2.80	138.0±3.29
c) Base nutrient+80 m.e. Na_2SO_4	142.7±3.65	172.3±2.71	126.5±2.44
d) Base nutrient+120 m.e. Na_2SO_4	135.4±2.67	170.3±2.86	118.3±1.73

* In any comparison, 14 degrees of freedom are available. When $t = 2.145$, $P = 0.05$ and when $t = 2.977$, $P = 0.01$.

practical purposes, the total growth depression produced by the addition of 120 milliequivalents of Na_2SO_4 to the base nutrient was the same in all three strains. If the concentrations of Na_2SO_4 had been applied earlier in the life of the plant, the magnitude of the differences might have been increased (11).

DRY WEIGHT OF PLANTS

On August 13, three plants of each strain grown in each nutrient concentration were selected for further experimentation. The selected plants were those which most closely approximated the strain mean for each treatment as regards plant height and date of first bloom. These plants were re-randomized and continued on their respective treatments. The remaining five plants of each strain in each treatment were harvested after all fruits, regardless of stage of development, had been picked. The root systems of each plant were washed successively through a series of mesh screens until separated from all sand particles. The vegetative parts (the fruits are not included) were dried at 80° C. and weighed (table 3).

It is possible to demonstrate in the data, mean differences of 35.2 ± 6.07 gm.,

95.4 ± 11.20 gm., and 42.8 ± 7.85 gm. for the vines of Johannisfeuer, F_1 generation, and Red Currant, respectively, when treatment *a* is compared with treatment *d*. The mean differences are well in excess of twice their standard error and are significant. In all strains the dry weight of the vines was less as the concentration of Na_2SO_4 in the nutrient solution was increased. When plotted against Na_2SO_4 concentrations, the dry weight of the vine closely approximated a linear regression in the case of the parental lines, while a comparatively pronounced reduction in dry weight was obtained for treatment *c* in the F_1 generation. The dry weight of vines in treatment *d* was 71, 69, and 56 per cent as great as in treatment *a* for Johannisfeuer, F_1 generation, and Red Currant strains, respectively. The effect

TABLE 3*

ANALYSIS OF DRY WEIGHT OF PLANTS GIVING STRAIN MEANS IN GRAMS TOGETHER WITH THEIR STANDARD ERRORS

Treatment	Johannisfeuer		F_1 generation		Red Currant	
	Vine	Root system	Vine	Root system	Vine	Root system
a) Base nutrient....	121.0±4.64	19.4±2.12	308.2± 7.67	62.9±3.67	97.8±6.10	18.0±2.61
b) Base nutrient+ 40 m.e. Na_2SO_4...	106.6±4.90	16.5±1.22	276.4± 7.06	57.3±2.01	83.8±2.60	18.7±0.63
c) Base nutrient+ 80 m.e. Na_2SO_4..	101.0±5.77	15.1±0.68	220.2±10.48	45.7±2.64	71.4±3.57	18.0±0.67
d) Base nutrient+ 120 m.e. Na_2SO_4.	85.8±3.92	14.9±0.67	212.8± 8.16	42.2±2.73	55.0±4.94	18.7±0.66

* In any comparison, 8 degrees of freedom are available. When t = 2.306, P = 0.05 and when t = 3.355, P = 0.01.

of a high concentration of Na_2SO_4 is relatively greater on the Red Currant strain than it is on the other two in this respect.

In a similar comparison of root systems (table 3), a mean difference of 4.5 ± 2.22 gm. and 20.7 ± 4.57 gm. in dry weight is obtained for Johannisfeuer and the F_1 generation, respectively, between treatments *a* and *d*. Within these two strains, correlation coefficients computed between the dry weight of the root system and that of the vine were found to be highly significant. (r = 0.73, t = 4.53 for Johannisfeuer; r = 0.80, t = 5.66 for the F_1 generation. Nineteen degrees of freedom are involved in each line.) This means that within the limits of precision obtainable in this experiment, the root system and vine growth of Johannisfeuer and the F_1 generation were affected in the same way and to much the same degree by increases in Na_2SO_4 concentration. In the case of the Red Currant strain, no statistically significant differences in the dry weight of the root system produced were demonstrable. This phenomenon will be discussed later.

The expression of heterosis is clearly demonstrable. The dry weight of the F_1 generation vines is approximately 2.5 times that of the Johannisfeuer strain, which

is the heavier of the parents, while root systems are approximately 3.0 times as heavy in a similar comparison. The relationship is apparently constant in all treatments. This agrees with the observations and data of POWERS (**18**).

FRUIT PRODUCTION

When the plants were harvested, all fruits—regardless of size or state or maturity—were picked, counted, and weighed (table 4). At that time a few isolated fruits of the F_1 generation were completely red. Complete color change had not occurred on any fruit in parental lines.

TABLE 4*

ANALYSIS OF FRUIT PRODUCTION GIVING STRAIN MEANS TOGETHER WITH THEIR STANDARD ERRORS

TREATMENT	JOHANNISFEUER			F_1 GENERATION			RED CURRANT		
	FRESH WEIGHT OF FRUIT (GM.)	NO. OF FRUITS	AVERAGE WEIGHT OF FRUIT (GM.)	FRESH WEIGHT OF FRUIT (GM.)	NO. OF FRUITS	AVERAGE WEIGHT OF FRUIT (GM.)	FRESH WEIGHT OF FRUIT (GM.)	NO. OF FRUITS	AVERAGE WEIGHT OF FRUIT (GM.)
a) Base nutrient	567±58.7	30±3.1	18.9±0.39	286±29.3	136± 7.9	2.1±0.16	6.9±2.00	45±12.2	0.16±0.016
b) Base nutrient+40 m.e. Na_2SO_4	542±63.8	34±3.9	17.1±2.93	174±37.2	113±12.9	1.5±0.16	9.9±2.30	65±15.3	0.16±0.013
c) Base nutrient+80 m.e. Na_2SO_4	529±79.7	36±2.7	14.8±1.49	144±14.9	108± 6.7	1.3±0.09	10.5±1.49	73± 9.4	0.14±0.008
d) Base nutrient+120 m.e. Na_2SO_4	342±61.8	33±4.8	10.2±0.88	105±17.9	90±11.8	1.2±0.08	10.2±2.66	81±16.9	0.12±0.012

* In any comparison, 8 degrees of freedom are available. When $t = 2.306$, $P = 0.05$ and when $t = 3.355$, $P = 0.01$.

The data for Johannisfeuer and the F_1 generation show mean differences in the fresh weight of the fruit produced of 225 ± 85.23 gm. and 181 ± 34.34 gm., respectively, when treatments *a* and *d* are compared. The differences are well in excess of twice their standard error and are significant. No statistically significant differences in this respect are demonstrable in the Red Currant strain. By comparing treatments *a* and *d*, the fresh weight of fruits produced by the Johannisfeuer are 40 per cent less as a result of the highest concentration of Na_2SO_4 used in the experiment. The fruit production of the F_1 generation was 63 per cent less in the same comparison. At every concentration of Na_2SO_4, greater effects were observed on the F_1 generation. The most pronounced were observed between treatments *c* and *d* in the Johannisfeuer strain and between treatments *a* and *b* in the F_1 generation. In other words, there are indications that differences within as well as between strains exist for this character in its response to high Na_2SO_4 concentrations.

The data for the number of fruits produced by the F_1 generation show a mean difference of 46 ± 14.2 when treatments *d* and *a* are compared. In this strain the high concentration of Na_2SO_4 had an inhibiting effect on the number of fruits pro-

duced. No statistically significant differences were demonstrable in this respect for parental lines.

By again comparing treatments *a* and *d* in respect to the average weight of the individual fruit produced, mean differences of 8.7 ± 0.96 gm. for Johannisfeuer and 0.9 ± 0.18 gm. for the F_1 generation are demonstrated. Both differences are highly significant. The data for the Johannisfeuer strain suggest that the effects of increasing Na_2SO_4 concentrations on fruit size are at least additive and possibly cumulative, while the major effect in the F_1 generation occurs between treatments *a* and *b*, where a significant mean difference of 0.6 ± 0.23 gm. is demonstrable. In both cases, however, the average weight of the fruit at this stage was less when Na_2SO_4 concentrations were increased. No statistically significant trends are shown in the data for the Red Currant strain. Possible interpretations of these data will be discussed later.

Inherent strain differences in the fresh weight of fruits produced at this time are apparent, as a mean difference of 560.1 ± 58.73 gm. is demonstrable between parental lines in treatment *a*. No mathematically significant differences are found in the data between the mean of the F_1 generation and the arithmetic or geometric means computed from the means of parental lines. In treatment *a* the Johannisfeuer and Red Currant strains do not differ significantly in respect to the number of fruits set at this time in their life cycle, but the F_1 generation set approximately three times as many fruits as the Red Currant, and hybrid vigor is evident. A highly significant mean difference of 18.7 ± 0.39 gm. in the average weight of the fruit produced is evident between parental lines. The observed mean of the F_1 generation is significantly lower than the arithmetic mean (9.5 ± 0.20) computed from the means of parental lines.

Mature fruit characteristics

The thirty-six plants (three replications, three strains, and four treatments) which had previously been selected for further experimentation on the basis of their uniformity and close approximation to the strain mean within treatments—in regard to plant height and date of first bloom—were grown until each plant had ripened twenty fruits. Each fruit was picked in the morning of the day that complete color change had occurred. Under these conditions, if the fruits are uninjured no detectable loss in weight occurs in the first 4 hours after harvest. Within this time limit each fruit was weighed in air to the nearest milligram and reweighed when immersed in distilled water. The weight in air is analogous to the mass (M) and the loss of weight in water is analogous to the volume (V). Fruit density (D) was computed by the equation $M/V = D$, using twenty fruits per plant and three plants of each strain in each treatment (table 5).

A mean difference in fruit density of 0.032 ± 0.0018 is indicated in the data for

parental lines in treatment *a*. The observed mean of the F_1 generation is significantly higher than either the arithmetic or geometric mean computed from parental strains, and partial dominance of the higher fruit density occurring in Red Currant is demonstrable. The relationship between strains is apparently constant in all treatments.

By comparing treatments *a* and *d*, mean differences in fruit density of 0.013 ± 0.0021, 0.013 ± 0.0024, and 0.017 ± 0.0012 for Johannisfeuer, F_1 generation, and Red Currant, respectively, were found. All differences are mathematically significant and the addition of 120 milliequivalents per liter of Na_2SO_4 to the base nutrient solution resulted in greater densities of mature fruits in all strains

TABLE 5*

ANALYSIS OF FRUIT DENSITY GIVING STRAIN MEANS TOGETHER WITH THEIR STANDARD ERRORS

Treatment	Strain		
	Johannisfeuer	F_1 generation	Red Currant
a) Base nutrient.....	0.996±0.0015	1.025±0.0019	1.028±0.0010
b) Base nutrient+40 m.e. Na_2SO_4.....	1.003±0.0001	1.026±0.0018	1.038±0.0011
c) Base nutrient+80 m.e. Na_2SO_4.....	1.005±0.0023	1.032±0.0028	1.039±0.0007
d) Base nutrient+120 m.e. Na_2SO_4.....	1.009±0.0014	1.038±0.0014	1.045±0.0007

* In any comparison, 118 degrees of freedom are available. When = 1.980, P = 0.05 and when t = 2.618, P = 0.01.

tested. No differences between strains were noted in the percentage increase in fruit density as a result of treatment *d*. When treatments *a* and *b* are compared, significant mean increases of 0.007 ± 0.0015 and 0.010 ± 0.0015 are demonstrable for the Johannisfeuer and Red Currant strains, while no statistically significant difference is noted in the F_1 generation. In this experiment the fruits of the F_1 generation were affected less by the addition of 40 milliequivalents per liter of Na_2SO_4 to the base nutrient than were the fruits of the parental strains.

The data were examined in regard to the mean weight of each fruit produced by the strains when each fruit is picked immediately after complete color change (table 6). In Johannisfeuer and the F_1 generation, mathematically significant mean differences of 19.0 ± 4.72 gm. and 1.6 ± 0.44 gm., respectively, are demonstrable by comparing treatments *a* and *d*. In both strains the weight of each fruit is significantly less in treatment *c* than in *a*, but the addition of 40 milliequivalents of Na_2SO_4 produced no significant effect. There are no significant trends in the data for the Red Currant strain.

In this experiment, in comparisons made within strains no detectable effect of increasing Na_2SO_4 concentration was obtained for the number of days from planting to the date when twenty fruits per plant were ripened. The increased salt concentration did not significantly affect either the period in days from first fruit ripe to twenty fruits ripe per plant or the period in days from first fruit set to first fruit ripe per plant. When it is recalled that the concentration of the nutrient solutions was increased by additions of Na_2SO_4 at or near the date of first bloom, no effects would be expected, nor were they observed, in number of days from planting to date of first bloom. Within strains, the period from first bloom to first fruit set was unaffected by the treatments. In other words, under the conditions of this

TABLE 6*

ANALYSIS OF AVERAGE FRESH WEIGHT OF MATURE FRUITS GIVING STRAIN MEANS TOGETHER WITH THEIR STANDARD ERRORS

Treatment	Strain		
	Johannisfeuer	F_1 generation	Red Currant
a) Base nutrient	57.6±4.07	4.1±0.35	0.84±0.064
b) Base nutrient+40 m.e. Na_2SO_4	52.0±3.58	4.9±0.37	0.75±0.056
c) Base nutrient+80 m.e. Na_2SO_4	42.1±3.03	2.8±0.33	0.80±0.042
d) Base nutrient+120 m.e. Na_2SO_4	38.6±2.39	2.5±0.26	0.84±0.031

* In any comparison, 118 degrees of freedom are available. When $t = 1.980$, $P = 0.05$ and when $t = 2.618$, $P = 0.01$.

experiment no detectable effects of increased concentrations on the rate of fruit development and maturation were observed.

The plants were harvested immediately after ripening twenty fruits per plant, and the dry weight of vines and root systems in all strains showed the same trends as in table 3. The dry weight of vines in all strains was less as the concentration of Na_2SO_4 was increased. The dry weight of the root systems produced by the Johannisfeuer strain and the F_1 generation was less as the concentration increased, but even at maturity no statistically significant differences in the dry weight of root systems in the Red Currant strain were demonstrable. As would be expected, the dry weight of the vines was greater in this harvest than in the earlier one, and the relative increase in dry weight of the vines was less as the concentrations of Na_2SO_4 increased. No statistically significant increase in the dry weight of root systems was shown in comparing data of both harvests. The magnitude of heterosis demonstrable in the dry weight of F_1 generation plants was less than in the previous harvest.

STEM ANATOMY

When each plant in the experiment was harvested, a section of the stem from the internode nearest the midpoint of the plant axis was fixed with Navashin's solution and air was evacuated from the tissues. The material was dehydrated in an ethyl-tertiary butyl alcohol series and infiltrated with a paraffin-beeswax-rubber mixture. Complete cross sections were cut at 15–30 μ and stained with a modified Flemming's triple stain. The material was mounted in balsam.

The stem sections of the plants which were harvested on August 13, as well as those harvested after twenty fruits were produced, had a continuous vascular cylinder, and comparatively few inner pericyclic fibers were thickened (**10**). The sec-

TABLE 7*

ANALYSIS OF STEM DIAMETERS GIVING STRAIN MEANS IN MILLIMETERS TOGETHER WITH THEIR STANDARD ERRORS

TREATMENT	STRAIN		
	JOHANNISFEUER	F_1 GENERATION	RED CURRANT
a) Base nutrient	15.0±0.47	14.2±0.53	9.6±0.31
b) Base nutrient+40 m.e. Na_2SO_4	13.3±0.41	13.1±0.34	9.1±0.25
c) Base nutrient+80 m.e. Na_2SO_4	11.5±0.38	12.4±0.41	8.2±0.23
d) Base nutrient+120 m.e. Na_2SO_4	10.7±0.31	11.2±0.31	8.2±0.22

* In any comparison, 4 degrees of freedom are available. When $t = 2.776$, $P = 0.05$ and when $t = 4.604$, $P = 0.01$.

tions were magnified 27.5 diameters and the diameter of each stem measured. Four diameters were selected in order to divide the section into eight equal sectors, and the mean of the four measurements was used for each stem. The data in table 7 pertain to those plants harvested immediately after ripening twenty fruits.

Mean differences between treatments *a* and *d* of 4.3 ± 0.56 mm., 3.0 ± 0.61 mm., and 1.4 ± 0.38 mm. are demonstrable for the diameter of the Johannisfeuer, F_1 generation, and Red Currant strains, respectively. All differences are of sufficient magnitude to provide "P" values as small as, or smaller than, 0.05 and are statistically significant. In all lines there was less increase in diameter as the concentration of Na_2SO_4 increased. The percentage diameter of the stem as a result of the addition of 120 milliequivalents per liter of Na_2SO_4 to the base nutrient solution was 71.3 per cent for Johannisfeuer, 78.9 per cent for the F_1 generation, and 85.4 per cent for Red Currant. The last strain was affected less in this respect than was the Johannisfeuer, while the magnitude of the effect on the F_1 generation was intermediate.

The data for those plants harvested on August 13, and hence of comparable age rather than comparable maturity, were similar in most respects to those just cited and are not reported. Similarly, a smaller diameter of the stem was demonstrable in all strains as a result of the high Na_2SO_4 concentration. The data differed, however, in that the effect of increased salt concentration on the F_1 generation was greater than it was on parental lines.

At the increased magnification, the tissue systems were measured to the nearest millimeter on eight radii separated from one another by an angle of 45°. The mean of eight radial dimensions for each tissue system was used in the computation of an actual area. The actual area was computed using standard formulas, which were developed for perfect circles. The data in table 8 pertain to those plants harvested after twenty fruits per plant had ripened. The standard errors provide an estimation of variability between plants.

There are no statistically significant differences within a strain in the percentage of area of any of the tissue systems. The smaller diameter of the stem when the concentration of Na_2SO_4 is increased is a direct result of a correspondingly smaller area (and diameter) of each of the constituent tissue systems. For instance, the actual area of the xylem in a cross section of the stem of Johannisfeuer was less as the concentration in the nutrient solution was increased, but no significant differences were obtained in the percentage of the area of the xylem in the cross section of the stem.

In the cross section of the stem, a significant mean difference of 19.7 ± 3.71 per cent is demonstrable in the percentage of actual area covered by the pith between parental strains. The F_1 generation is intermediate between the parental strains in this respect, and differs significantly from both. The amount of cambial activity differs between parental lines when the formation of secondary xylem and phloem tissues is used as a criterion. A mean difference of 17.7 ± 3.37 per cent is shown for the amount of secondary xylem formed, and a mean difference of 3.8 ± 1.09 per cent is demonstrable for the secondary phloem. Both mean differences are statistically significant, and in both respects the F_1 generation is intermediate between parental strains. No significant difference is demonstrable in the percentage of area covered by the cortex. The cross section of the stem of the Johannisfeuer strain has a stele with a comparatively large amount of pith and small amounts of vascular tissue, while the stele of Red Currant has comparatively large amounts of vascular tissue and a small amount of pith. The stele of the F_1 generation is intermediate in all respects. The addition of increased concentrations of Na_2SO_4 to the nutrient solution does not alter inherent differences between genotypes in respect to the anatomy of stem sections.

The stems of the plants harvested when 87 days old showed the same anatomical relationships as those harvested after ripening twenty fruits per plant. The

TABLE 8*

ANALYSIS OF COMPOSITION OF STEM CROSS SECTIONS GIVING STRAIN MEANS IN PERCENTAGE OF ACTUAL AREA TOGETHER WITH THEIR STANDARD ERRORS

Treatment	Percentage area											
	Johannisfeuer				F_1 generation				Red Currant			
	Pith	Xylem	Phloem	Cortex	Pith	Xylem	Phloem	Cortex	Pith	Xylem	Phloem	Cortex
a) Base nutrient	43.5±3.28	27.7±3.19	5.7±0.30	19.3±0.51	34.3±2.09	36.2±1.19	7.4±0.61	20.1±1.31	23.8±1.73	45.4±1.07	9.5±1.05	17.3±1.20
b) Base nutrient +40 m.e. Na_2SO_4	39.2±1.92	32.1±1.95	6.0±0.57	22.2±2.65	32.6±5.56	37.5±4.10	8.1±0.34	19.1±1.33	24.8±3.33	45.5±2.40	9.9±1.98	16.6±0.29
c) Base nutrient +80 m.e. Na_2SO_4	44.0±2.50	29.1±2.14	6.0±0.31	18.6±0.78	32.7±0.50	38.1±0.38	6.1±0.37	18.9±0.79	24.0±1.96	48.5±1.55	8.8±0.61	17.0±0.71
d) Base nutrient +120 m.e. Na_2SO_4	42.5±2.03	31.9±2.08	6.1±0.40	16.9±1.07	29.6±3.00	39.8±2.00	8.1±0.27	20.1±1.91	24.1±2.46	48.0±1.12	9.2±1.43	16.4±0.60

* In any comparison, 4 degrees of freedom are available. When $t = 2.776$, $P = 0.05$ and when $t = 4.604$, $P = 0.01$.

magnitude of the differences was less at this date, but the differences were statistically significant. No effects of treatment were noted in the data.

Discussion

The growth depressions produced as a result of growing plants in nutrient solutions containing high salt concentrations have been pointed out (**1, 17**). EATON (**6**) and HAYWARD and LONG (**11**) have shown differential ionic effects at isosmotic concentrations of the nutrient solution. Unpublished data collected by EATON have indicated an interaction between a given salt concentration and such factors of the environment as temperature. It seemed logical that attention should also be given to the hereditary qualities of the test plant in addition to the preceding considerations.

Two criteria were used to measure the relative responses of the plants. First, at a given age, what is the response of the plant to varied environments with respect to any character? This method has been widely used in both physiological and genetic studies. Second, at comparable "physiological maturity," what is the response of the plants to varied environments in respect to any character? It was arbitrarily considered in this paper that plants were at comparable stages of physiological maturity when each had matured and ripened twenty fruits. The latter method has been less extensively used.

Where statistical significance is not demonstrable in an experiment (as is the case in the data for the Red Currant strain) in respect to mean weight of each ripe fruit produced, dry weight of the root system, total fresh weight of immature fruits, etc., it does not mean that differences do not exist. The use of larger populations and more refined methods might demonstrate significant differences in these characteristics. It may be assumed, however, that in this experiment Red Currant was less affected in these characteristics by high concentrations of Na_2SO_4 than were the other two strains.

The results of this experiment have shown that the presence of high concentrations of sodium sulphate in the nutrient solution affects the growth of all three strains. In many respects, the effects produced by the various concentrations on the strains were very similar. For example, the phenomenon of heterosis exhibited by the hybrid with respect to growth in height, dry weight production, number and weight of fruits, and so on, was exhibited to almost the same degree at every salt concentration used. In comparing the effects of increasing the concentration of sodium sulphate in the nutrient solution on the individual strains, it may be noted that Red Currant was affected less than were the other two with respect to such characters as dry weight of root system, fresh weight of all fruits produced, and average weight of mature fruit. For these characters the F_1 hybrid paralleled the Johannisfeuer parent more closely than it did the Red Currant. In all three

strains the density of the ripe fruit produced by plants subjected to increasing concentrations of sodium sulphate increased. Just what effect these changes in density might have upon the nutrient value of the fruit is not known. The effects of presence of sodium sulphate in the solution upon the internal anatomy of the stem of the three strains are very similar. In general there was no indication that the presence of sodium sulphate had a differential effect on any tissue system. In every case, the higher the concentration the smaller was the stem diameter; and in all stems the relative proportions of pith, xylem, phloem, and cortex remained the same, within the limits of error.

Anatomical results are not in entire accord with those of HAYWARD and LONG (11). There are, however, several differences in the methods of experimentation, such as: (1) the time at which the plants were subjected to the various concentrations in Na_2SO_4, (2) ionic concentrations of nutrient solutions used, (3) pH of the solutions, (4) environmental factors, and (5) hereditary qualities of the plant. It is possible that future work may correlate the differences in the results obtained with one or more of these factors.

The occurrence of saline areas is general throughout western states. In addition, comparatively high concentrations of Na_2SO_4 and other salts are prevalent in water supplies used for irrigation purposes. To accompany proper soil management practices and more detailed studies of salt antagonism and toxicity effects, it may be possible and desirable to select and breed plants for tolerance to saline conditions. Such a program should eventually involve not only the production of strains with the characteristic of general tolerance to high salt concentrations but the actual selection and breeding of strains for specific concentrations of two or more ions.

Summary

Tomato plants of the Johannisfeuer strain and the Red Currant strain, together with the F_1 generation, were submitted to four treatments, providing four concentrations of Na_2SO_4 ranging from 4.6 to 124.6 milliequivalents of sulphate ion per liter of nutrient solution.

1. Plant height was less in all strains as the concentration increased. The growth depression resulting from the highest concentration was the same in all strains.

2. The dry weight of vines was less in all strains as the concentration increased.

3. The dry weight of the root system of the Johannisfeuer strain and the F_1 generation was less when the concentration was increased. No statistically significant reduction in this respect was obtained for the Red Currant strain.

4. The total fresh weight of immature fruits produced by plants 87 days old, as well as the average weight per fruit, was less in two strains when salt concentration was increased. No differences were noted in the Red Currant strain.

5. The number of immature fruits produced by each plant of the F_1 generation was less as the concentration increased.

6. The density of ripe fruits increased in all strains with increased concentration.

7. The mean weight of each ripe fruit of the Johannisfeuer strain and the F_1 generation was less with increased concentration.

8. The diameter of the stem of plants harvested after ripening twenty fruits, as well as of plants harvested at an earlier date, was less in all strains with increased concentration.

9. The smaller stem diameter was caused by an inhibited development of each of the component tissue systems.

10. Inherent differences between strains are discussed for each of these characters.

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SEROLOGICAL STUDIES OF THE ERWINEAE
I. ERWINIA AMYLOVORA

R. P. ELROD

Introduction

Erwinia amylovora (Burrill) Winslow *et al.*, etiological agent of fire blight, has been considered by some to be a well-defined species, yet others have found that various isolates often show numerous minor cultural and morphological differences. These dissimilarities have never been considered great enough to warrant new varieties, although they are often constant and readily distinguish one isolate from another (**1, 11, 12, 16, 25, 19**).

Serological methods have been employed with only moderate enthusiasm for bacterial plant pathogens, but enough work has been done to bring out the fact that certain species, such as *Phytomonas malvacearum* (E.F.S.) Bergey *et al.* (**10, 26**), *P. pisi* (Sacket) Bergey *et al.* (**23**), and *P. tabaci* (Wolf and Foster) Bergey *et al.* (**21**) are apparently serologically homogeneous, while others such as *P. tumefaciens* (E.F.S. and Townsend) Bergey *et al.* (**20**) and *P. stewarti* (E.F.S.) Bergey *et al.* (**2, 15**) are heterogeneous from an antigenic standpoint. In view of the increasing importance attached to the application of serological methods, it would be desirable to ascertain whether various isolates of *E. amylovora* might be distinguishable by agglutination, agglutinin-absorption, and also by precipitin tests, the last to be performed with carbohydrate materials extracted from the bacillary cells. A preliminary report of the present work has already been made (**5**).

Material and methods

The nine cultures[1] employed were obtained from widely separated localities and in the majority of cases from different hosts. The first four have been well discussed by ARK (**1**). His work gives the numerous differences detected in these isolates.

The sources of the nine isolates are as follows:

50.—Isolated from a stem canker on *Pyracantha angustifolia* at Berkeley, California, August, 1929.

55.—Isolated from a berry of *P. angustifolia* at Berkeley, January, 1932.

501.—Isolated from a twig lesion on *Crataegus oxyacantha* at Ithaca, New York, July, 1931.

SC.—Isolated from a twig lesion on *Pyrus communis* in South Carolina, June, 1925.

[1] The writer is indebted to Drs. P. A. ARK, E. L. WALDEE, E. M. HILDEBRAND, and S. B. DOOLITTLE for supplying some of the cultures used.

37.—Isolated from apple twig in Wisconsin, 1937.
84.—Isolated from *Crataegus monogyra* at Ames, Iowa, 1938.
83.—Isolated from *P. communis* at Ames, Iowa, 1938.
AM.—Obtained from Dr. E. M. HILDEBRAND, Ithaca, New York.
37, 83, 84, 85.—Obtained from Dr. E. L. WALDEE at Ames, Iowa.

Isolates 50 and SC were found by ARK to be only weakly pathogenic; all the others were moderately to strongly virulent. It was impossible for this writer to make virulence tests, but in view of ARK's observation that cultures of this organism will remain virulent after many years on stock agar, it can be assumed that in this test there were both virulent and avirulent isolates.

In the present work each isolate was streaked on nutrient agar, and from each plate a well isolated-smooth colony was picked and transferred to agar slopes containing nutrient agar having an agar concentration of 1.3 per cent. The growth from such a moist medium has been found to yield bacillary suspensions of high antigenic value. This growth was harvested with sterile saline solution and the cells were packed in an angle centrifuge and resuspended in saline in such a manner that the suspension contained approximately four billion bacteria per milliliter.

Rabbits weighing approximately 5 lb. were used. The following schedule of injections was employed: first day, 0.1 ml.; second day, 0.2 ml.; third day, 0.3 ml.; eighth day, 0.5 ml.; and thirteenth day, 1.0 ml. For the fourth and fifth injections new suspensions were prepared. All injections were made intravenously. The animals were bled by cardiac puncture on the eighteenth day and the sera preserved with merthiolate (1:10,000). Antisera were prepared for five of nine isolates. In all cases high agglutinating sera were obtained, the homologous titers ranging from 5,120 to 20,480.

Agglutination tests were set up in 0.5-ml. serial dilutions, beginning with 1:40 and carried past the titer of the serum, usually to 1:80,000. Antigen (0.05 ml.), freshly prepared as described, was added to each tube. The tubes were incubated in a waterbath for 4 hours at 50°–52° C. and placed overnight in the icebox (5° C.). The titer was determined as that tube with the highest dilution of serum which manifested clumping determinable by the naked eye. Saline and normal serum controls were used in each experiment. The latter was drawn from the rabbits before the beginning of the immunization series. In all cases controls were negative. Tests also were carried out, using as an antigen the primary suspension employed for the first injection, to which 1 per cent formalin had been added. No significant differences were noted except an enhancement in the degree of flocculation with the formalinized suspensions.

For the preparation of antigens for the absorption experiments, care was taken to select smooth colonies. These were picked into nutrient broth, and after 24 hours nutrient agar plates (1.3 per cent agar) were streaked heavily with a sterile

cotton swab which had been dipped into the broth culture. After 36 hours of incubation at room temperature, the growth was harvested by washing the organisms from the plate with sterile saline solution. The growth of three plates was combined and spun sharply in an angle centrifuge (3500 r.p.m.). After the cells were packed all excess moisture was drained from them. The quantity of packed cells was in the neighborhood of 0.25–0.4 ml. Ten ml. of anti-serum (1:20) was then added to the cells, the tubes were shaken until there was an even turbidity, and then incubated 1 hour at 37° C. in a waterbath. The suspension was centrifuged and the supernatant liquid decanted to another portion of packed cells.

TABLE 1

CROSS-AGGLUTINATION TITERS OF ERWINIA AMYLOVORA ISOLATES*

ORGANISM AGGLUTINATED	ANTI-SERUM PREPARED AGAINST				
	50	501	AM	55	84
50	5,120	20,480	10,240	20,480	10,240
SC	2,560	20,480	5,120	20,480	40,960
501	5,120	20,480	10,240	10,240	20,480
55	5,120	10,240	20,480	20,480	20,480
AM	10,240	20,480	10,240	20,480	20,480
37	10,240	10,240	20,480	10,240	20,480
84	5,120	10,240	10,240	10,240	20,480
83	10,240	10,240	5,120	10,240	20,480
85	5,120	10,240	10,240	10,240	10,240

* Saline and normal serum controls negative.

Usually it was necessary to repeat the procedure four times. The fourth absorption was incubated 2 hours at 37° C. and then placed overnight in the icebox. After centrifuging, the supernatant was diluted to 1:40. Further serial dilutions were made as found necessary.

Experimental results

AGGLUTINATION TESTS

Agglutination tests were performed with the five sera, using suspensions of the nine isolates as antigens. Table 1 shows the high degree of antigenic homogeneity. The homologous titers ranged from 5,120 to 20,480, and the cross-agglutination titers were virtually as high. In some cases the latter dropped one tube below the homologous titer, as for example culture SC (2,560) in anti-50 serum (homologous titer 5,120), and in some cases they advanced to as great a degree (the homologous titer anti-AM serum was 10,240; the heterologous 55 was 20,480). It would be impossible, however, to distinguish one isolate from another on the basis of these reactions.

The type of agglutination was extremely variable. All the isolates proved to be peritrichously flagellate, as stained by the method of LEIFSON (**14**). This flagellation was emphasized by the usually typical floccular type of clumping. On occasion, however, this type of agglutination was less evident and the somatic or granular type was predominant. The addition of 1 per cent formalin to the antigen tended to stabilize the type of agglutination and usually enhanced the floccular type.

That this high degree of cross-agglutination was specific for *E. amylovora* was brought out by cross-agglutination tests performed with four cultures of *E. tracheiphila* (E.F.S.) Holland (isolates 71A, 72A, 73B, and 75C), one of *E. salicis* (Day) comb. nov. (isolate 80), one of *E. lathyri* (Manns and Tabenhaus) Magrou

TABLE 2

CROSS-AGGLUTINATION TITERS WITH UNRELATED ORGANISMS*

ORGANISM AGGLUTINATED	ANTI-SERUM PREPARED AGAINST				
	50	501	AM	55	84
71A	<40	160	80	40	160
72A	<40	< 40	< 40	<40	< 40
73B	<40	< 40	< 40	<40	< 40
75C	<40	160	160	40	80
80	<40	80	< 40	40	80
BL	<40	< 40	< 40	<40	< 40
MY	<40	< 40	< 40	<40	< 40
20 strains soft-rot organisms	<40	< 40	< 40	<40	< 40

* Saline and normal serum controls negative.

(isolate BL), one of *E. ananas* Serrano (isolate MY), and twenty strains of organisms of the soft-rot group. As can be seen in table 2, cross-agglutination occurred in only a small percentage of the tests. Whether or not the cross-reactions of certain strains of *E. tracheiphila* and *E. salicis* are only a unilateral relationship has not been ascertained. This relationship will be examined in subsequent experiments. In no case, however, did these titers approach the homologous reactions, and for the most part did not exceed 1 per cent of the homologous titer.

AGGLUTININ-ABSORPTION EXPERIMENTS

The results of the agglutinin-absorption experiments are recorded in table 3. All five sera were absorbed with each of the nine isolates, forty-five absorptions in all. In each case a complete removal of homologous and heterologous agglutinins resulted. It may be assumed, therefore, that at least five of the isolates (50, 501, 55, AM, and 84) are serologically identical (those for which anti-sera were pre-

pared), and that the other four contain at least those fractions common to the preceding five. Whether or not they possess other antigenic factors could be determined only after preparing anti-sera for them. There is no doubt, however, that the nine isolates are closely related, if not serologically identical.

The experiments were controlled for specificity by absorbing each of the sera with an unrelated organism (for example, *E. tracheiphila* or *E. salicis*). In no case were any agglutinins removed, either homologous or heterologous.

Precipitin tests

Since Dochez and Avery (**4**) discovered substances in pneumococcus filtrates which were later shown to be carbohydrate in nature (**9**), bacterial polysaccharides have become an important factor in problems concerned with bacterial classification. Specific carbohydrates have been isolated from many groups of bacteria. Such work with the *Salmonella* group (**7, 8**), hemolytic streptococci (**13**), and other forms have been landmarks in the progress of bacteriology.

TABLE 3

Absorption experiments

Anti-serum	Absorbed by	Residual agglutination against all strains
50	50, 501, 55, AM, SC, 83, 84, 85, 37	Nil*
501	501, 50, 55, AM, SC, 83, 84, 85, 37	Nil
55	55, 501, 50, AM, SC, 83, 84, 85, 37	Nil
AM	AM, 501, 50, SC, 83, 84, 85, 37, 55	Nil
84	84, AM, 501, 50, SC, 83, 85, 37, 55	Nil
50, 501, 55, AM, and 84	E. tracheiphila or E. salicis	All to titer (table 1)

* Nil = less than 1:40.

In view of the success attained in other groups, it was deemed advisable to ascertain whether the isolates of *E. amylovora* could be distinguished one from another by means of precipitin tests, using extracted carbohydrate materials as antigens. Previous precipitation experiments with phytobacteria have employed filtrates of broth cultures. Such an antigen certainly cannot always be considered specific but will contain carbohydrates and protein as well as metabolic products.

The method used for extraction was that of Fuller (**6**). It was found admirable for the purpose, although it was usually shortened by eliminating the acid-alcohol step. The material thus obtained gave a strongly positive Molisch test for carbohydrate and a weak or negative Biuret reading for protein. For the precipitin tests the material was serially diluted and ring tests employed in the usual

manner. The tests were read after 1 hour of incubation at room temperature. The titers were all consistently the same (1:100,000).

As shown in table 4, it was impossible to distinguish one isolate from another by means of direct precipitin tests. It is possible that absorption experiments with carbohydrate material might detect differences. Absorption with whole cells resulted in negative results, however, even as had the agglutinin-absorption experiments. Carbohydrates derived from *E. salicis* (isolate 80) and *E. tracheiphila* (isolate 75C) were negative in all immune sera. This was likewise true of saline controls. The various *E. amylovora* antigens failed to precipitate in normal serum.

TABLE 4

PRECIPITIN TESTS WITH EXTRACTED CARBOHYDRATES

ANTIGEN (1–100,000)	ANTI-SERUM PREPARED AGAINST					NORMAL SERUM
	50	501	AM	55	84	
50	+	+	+	+	+	–
501	+	+	+	+	+	–
AM	+	+	+	+	+	–
SC	+	+	+	+	+	–
55	+	+	+	+	+	–
83	+	+	+	+	+	–
84	+	+	+	+	+	–
85	+	+	+	+	+	–
37	+	+	+	+	+	–
E. salicis (1:100)	–	–	–	–	–	–
E. tracheiphila (1:100)	–	–	–	–	–	–
Saline	–	–	–	–	–	–

Discussion

In general, the bacteriologist has had little success in attempting to link source of isolation, virulence, physiology, and morphology with definite serological results. In the realm of phytobacteriology, BUSHNELL and SARLES (3) noted that cross-inoculation experiments and cross-agglutination experiments with root-nodule bacteria could not be correlated. This had been previously found to be true by STEVENS (24). Likewise, BRAUN and MCNEW (2, 15) found that differences in serological properties could not be correlated with virulence or colony type in *Phytomonas stewarti*. SHARP (18) concluded that two strains of *Bact. flaccumfaciens*, although differing in virulence, type of colony produced, and appearance on agar slopes, were not distinguishable by agglutination experiments. This was also true of *Bact. phaseoli* (18).

The results presented in this paper show that serologically *E. amylovora* is a homogeneous species, and that no groups are demonstrable which might be cor-

related with source of isolation, morphology, and physiology. In this case, however, instead of there being several serological groups in which cultural and morphological differences overlap, we are dealing with a serologically constant group, in which there is wide variance in morphology, physiology, and pathogenicity (1).

The precipitation experiments reported in this paper were not concerned with showing a group relationship, but were an attempt to isolate a specific carbohydrate. STAPP (22) contends that when a positive precipitation test is obtained the identity of the species is confirmed, no matter what the cultural and morphological characteristics might be. This is in all probability true with *E. amylovora*, but offers no advantages over the more easily performed agglutination test. Inasmuch as there are no means of differentiating the isolates serologically, one from another, it is impossible to conclude whether the carbohydrate material is group or type specific in nature. That it could be one or the other is shown by the fact that in *Salmonella* (8), carbohydrate material derived from the bacillary cell acts in the same manner as the somatic components, that is, they are group specific. In *Proteus* (17) such carbohydrate material confers type specificity to the organism.

An absolutely serologically homogeneous species of bacteria is not in the least common. Numerous well-defined species often segregate themselves into many serological groups. Among the bacterial plant pathogens, *Phytomonas tumefaciens* (20), *P. stewarti* (2, 15), and others act in this manner. It is difficult to understand why *E. amylovora* should show such homogeneity, although it is true that certain other phytobacteria show similar serological characteristics (10, 26, 23, 21), but not in the striking manner demonstrated by *E. amylovora*.

It may be that *E. amylovora* represents the ultimate in a definite evolutionary chain, and that owing to a specialized pathogenic action within a limited host range the organism has become uniform. The arguments against such being the case are numerous and the idea is presented only as a possibility. For the supposition to obtain credence it would be necessary to demonstrate other links in the chain. None is at present known. The fact that my cultures had been carried in stock for many years might account in some way for this homogeneity. Perhaps isolates taken directly from the field would show some antigenic variation. This is only a bare possibility, however, for many organisms closely related serologically (for example, *Salmonella*) do not become serologically one after years in stock.

Summary

1. Nine isolates of *Erwinia amylovora*, obtained from five different localities and from as many different hosts, were compared serologically.

2. On the basis of agglutination tests and agglutinin-absorption experiments, no antigenic difference among any of the nine isolates could be detected.

3. Carbohydrate materials extracted from the bacillary cells and used in precipitation tests likewise failed to differentiate the organisms.

4. It is concluded that serologically *E. amylovora* is an exceedingly homogeneous species.

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HISTOLOGICAL STUDIES ON THE ROOT OF MELILOTUS ALBA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 527

FRANCES R. BOTTUM

(WITH FOURTEEN FIGURES)

Introduction

The distribution, soil adaptation, habits, and agricultural value of biennial white sweet clover, *Melilotus alba* Desv., have been extensively investigated, a notable survey being that of LLOYD (7). Studies of the relative growth rates of roots and tops have been made by SNIDER and HEIN (11), WILLARD (15), and MARTIN (9). MARTIN has also described the germination and establishment of the seedling. The habit of growth of the root system has been investigated by WEAVER (14). COOPER (1), GUIGNARD (4), WATT (13), and YOUNG (20) have given accounts of the embryogeny; and the cytology of the meristematic cells of the root tip has been investigated by ELDERS (2). MCMURRY and FISK (10) have reviewed those points in earlier studies of seedling structure in the Leguminosae by DE CANDOLLE, VAN TIEGHEM, NÄGELI, GERARD, and COMPTON which are of significance in the study of *Melilotus*, and they have described the transition of root to stem structure in the seedling.

The object of this investigation is to supplement these studies with a description of further details of the root structure, especially of the older roots.

Material and methods

The plants used in this study were grown in the field, except very young seedlings for which pots of soil or germination dishes were used. The seed coats were softened by treatment with concentrated sulphuric acid, employing the method of LOVE and LEIGHTY (8), except that the time was shortened from 15 to less than 5 minutes.

Inoculation of soil with nodule-forming bacteria was insured by mixing the seeds, before sown, with fresh soil taken from around the roots of healthy older plants growing elsewhere. Plants were collected at convenient intervals throughout the period of growth.

Whole seedlings and short pieces of older roots were killed and fixed in formalin-acetic-alcohol or in Navashin's solution. The methods of clearing seedlings and free-hand sections in chloral hydrate or in cedar oil, of maceration, and of imbed-

ding in paraffin were used. Xylol was the clearing agent employed, and sections of roots were made at 8 or 12 μ. They were stained with Flemming's triple stain or with safranin and fast green.

Observations

Primary root of seedling

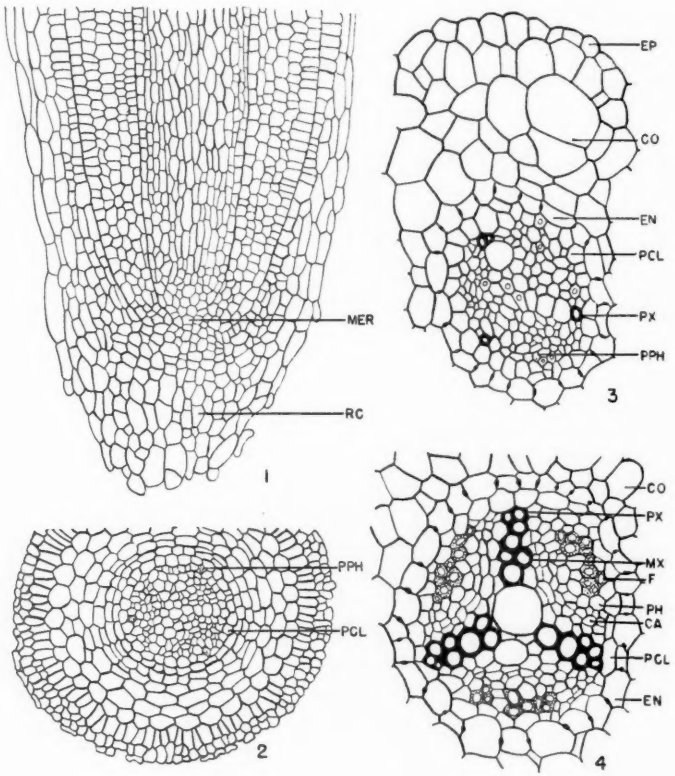
In the embryo of the seed, according to Martin (9), the radicle, hypocotyl, cotyledons, plumule, and even the crown buds are recognizable. In germination of the seed, the primary root on emerging penetrates the soil and forms a slender unbranched tap root. The ontogeny of the primary root corresponds to the fourth angiospermous type as described by Janczewski (5). Growth is accomplished by the activity of a transverse plate of meristem from which the stele, cortex, and central cone of tissue of the root cap develop. A lateral meristem, continuous near the root tip with the terminal meristem, gives rise by radial and periclinal divisions of the cells to the outer portion of the calyptra, and proximally constitutes a distinct dermatogen (fig. 1).

The root cap is composed of closely packed cells with conspicuous nuclei and relatively dense cytoplasm, except in the outer layers of cells which are separating from the cap. The young root immediately proximal to the terminal meristem shows a central region of small cells, varying in size, surrounded by one or two sheaths of larger cells, many of which are undergoing conspicuous tangential division and are densely cytoplasmic. Outside this central core is a peripheral region of large cells with deeply staining nuclei and dense cytoplasm, and the sheath of dermatogen with a few loosened layers of root-cap cells. There are no clearly defined plerome and periblem.

The first discernible differentiation of stelar tissue is the very early appearance of three protophloem strands abutting a well-defined uniseriate pericycle (fig. 2). These alternate with ridges of a triradiate central mass of larger parenchymatous cells, many of which later constitute the primary xylem. The cells of the protophloem are conspicuous because of their radial arrangement, the thinness of their cytoplasm, and the fact that their lateral walls are compressed by the adjacent meristematic cells. In longitudinal sections they appear to have elongated while adjacent cells continued to divide. The end walls are not tapering and there are no sieve plates. They retain their identity until after the protoxylem is differentiated. At the level of first appearance of the protophloem, the outermost layers of cortical cells become spherical, and intercellular spaces appear. The epidermis becomes clearly defined by the smaller size, long radial axis, and compactness of its cells.

In the region of most rapid elongation the metaphloem is differentiated, the sieve tubes developing conspicuous slime plugs very early. Companion cells ap-

pear at the same time but fibers are differentiated slightly later. Root hairs are not yet formed in this region but first appear where differentiation is most active. They are not confined to a definite zone but persist on all roots until the epidermis



FIGS. 1–4.*—Primary root of seedling: Fig. 1, longisection of root tip. Fig. 2, transection showing differentiation of protophloem and pericycle. Fig. 3, same at level of differentiation of protoxylem and endodermis. Fig. 4, same at level of completion of primary differentiation and formation of cambium.

* Symbols in all figures: *b*, tissue infected with bacteria; *ca*, cambium; *co*, cortex; *en*, endodermis; *ep*, epidermis; *f*, fiber; *mer*, meristem; *mx*, metaxylem; *pcl*, pericycle; *pd*, periderm; *ph*, phloem; *pi*, pith; *pph*, protophloem; *px*, protoxylem; *r*, ray; *rc*, root cap; *trc*, temporary root cap; *v*, vascular strand; *xy2*, secondary xylem.

is lost. Near the level of formation of the youngest root hairs the endodermis and protoxylem ridges are differentiated (fig. 3). The former consists of a single layer of the innermost cortical cells adjacent to the pericycle and having narrow Casparian bands. Each protoxylem ridge consists of one to four adjacent spiral or annular vessels, the outermost being smaller and next to the pericycle. The primary

root is triarch most commonly, but is not infrequently tetrarch, as shown by McMURRY and FISK (10) in their figures illustrating the condition of the tap root at the close of primary differentiation.

Whereas the outermost phloem cells differentiate as fibers first, sometimes giving the appearance of six bundles of fibers, others may soon form between them from parenchymatous cells adjacent to the pericycle, so that the whole phloem mass is frequently capped by fibers. Parenchyma, usually one or two layers of cells, separates the phloem and xylem. In this the cambium later arises by divisions tangential to the metaxylem (fig. 4).

Lignification of the metaxylem elements, which consist of cells with reticulate or simple-pitted walls, proceeds centripetally to include most of the central cells of the stele, though frequently some of these remain parenchymatous. Occurrence of an unusually large central metaxylem vessel is not uncommon.

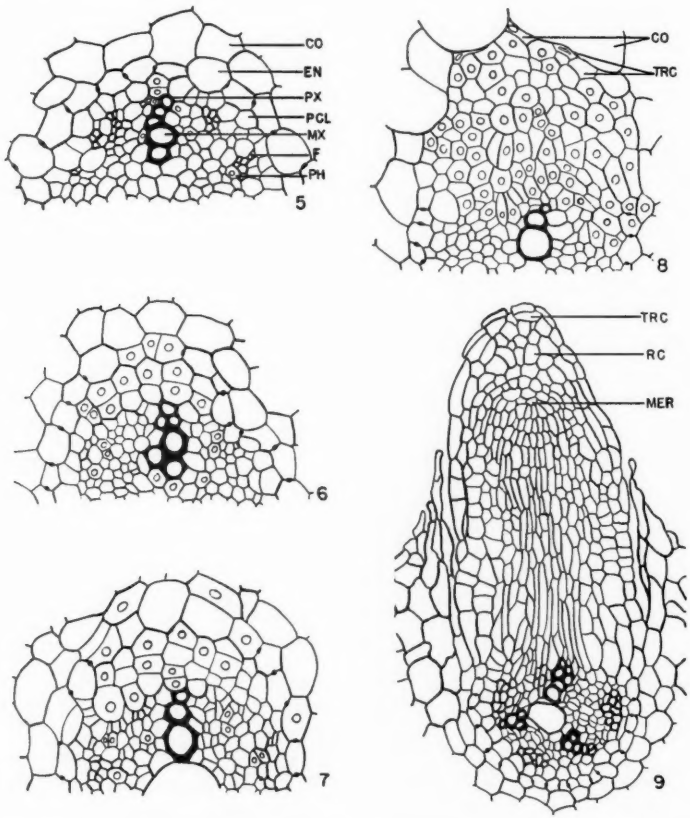
SECONDARY ROOTS

Before initiation of secondary growth and near the close of primary differentiation in the young tap root, lateral roots develop. These start growth through the periclinal divisions of a single pericyclic cell next to a protoxylem vessel, followed by similar divisions of a plate of adjacent pericyclic cells. Two successive periclinal divisions occur, followed by radial divisions and by stretching and enlargement of the overlying endodermal cells (figs. 5–7). As growth by further activity of pericyclic cells proceeds, the endodermal cells next to this new tissue likewise divide radially and tangentially, forming a temporary root cap at the tip, two or three cells in thickness. Most of the temporary cap cells are crushed against the cortical cells of the older root, are digested, or—after emergence of the root tip—are worn off or crushed by the soil, The cortical cells in turn are crushed, digested, or torn and pushed aside mechanically by the emerging root (figs. 8, 9).

The secondary roots develop a vascular system in a manner similar to that of the primary root. They are triarch usually, sometimes diarch, with a conspicuous endodermis.

During the third and fourth months, by which time a large amount of secondary tissue has developed near the crown of the plant, secondary roots arise in abundance on older roots and on the hypocotyl. Although the first node above the hypocotyl and the bud in the axil of the unifoliate leaf may become buried by soil and litter and remain alive during the winter of the first year, no adventitious roots were observed to arise above the first node. Adventitious and secondary roots, arising late in ontogeny, appear always opposite the primary rays, singly or in groups of two or three, emerging often close to the stump of an earlier rootlet which has died. The emergence of such roots is especially conspicuous during late autumn and winter months. Their formation begins in very early summer, when

the diameter of the primary root scarcely exceeds 1 mm., so that when the crown buds of the second year begin active growth, these roots have themselves branched and bear root nodules.



FIGS. 5–9.—Development of secondary root: Fig. 5, transection of portion of primary root showing initiation of secondary root by division of single pericyclic cell. Fig. 6, same showing division tangentially of plate of pericyclic cells and radial division of endodermal cell. Fig. 7, same showing second tangential division of pericyclic cells and tangential division of endodermal cells. Fig. 8, longisection of young branch root showing crushing and digestion of cells of cortex of primary root and of temporary root cap. Fig. 9, transection of primary root showing emergence of secondary root.

The adventitious roots arise by meristematic activity of the cork cambium and underlying pericyclic cells. The first vascular tissue of the young root differentiates through derivatives of the ray to that of the older root. The outer and more distal parts of the adventitious root are derived from the periderm, and its vascu-

lar system is later continued by differentiation of cells of its own histogen. As the adventitious root emerges, its base is surrounded by a glistening white mass of cells, formed by the cork cambium, which continues as a thin sheath over the root tip. The sheath is finally crushed, its cells disintegrating and leaving the young root free.

Subsequent development is not unlike that of other roots. The endodermis is more conspicuous than it is in the tap root. The adventitious roots are diarch or triarch most commonly, though tetrarch—and rarely pentarch—roots were found.

Secondary structures

Cambial activity in the primary root begins when the seedling is 7–8 days old (10). During the third and fourth weeks of growth of the seedling and before the first trifoliate leaf has appeared, the cambium, at first limited to cells between the metaxylem and phloem, comes to involve the cells of the pericycle which are adjacent to the protoxylem. This starts the development of the primary rays and completes the cambium cylinder as secondary growth proceeds. At this stage numerous secondary roots have appeared and attained lengths of 1–2 mm., root nodules have developed, and the tap root is about 3 inches long. Root hairs cover the entire root system except the tips distal to the region of differentiation. The cork cambium has arisen in the endodermis.

During the second month several trifoliate leaves appear, and the hypocotyl begins to withdraw by contraction underground. The tap root attains a length usually exceeding 1 foot, and rootlets of the third order have appeared. Root hairs persist except in the upper part of the tap root where they have been lost with the rest of the epidermis after differentiation of the periderm.

By the end of the fourth month the tap root may have become several feet in length. Two or three of the lateral roots have developed into large branches, nearly equaling the tap root in diameter at the level of their divergence and in depth reached in the soil. Most of the lateral roots, however, remain short. At this time the largest diameter of the tap root about equals that of the hypocotyl and stem.

Throughout the late spring and summer of the first season a large body of vascular tissue develops, parenchyma remaining relatively inconspicuous. Transverse sections of the tap root show a compact mass of primary and secondary xylem and numerous narrow rays. The primary phloem persists, as yet uncrushed by secondary growth. The periderm is about twelve cells in thickness, and its outer suberized cells have sufficiently thick walls to show pits distinctly.

Cells of the vascular cambium are arranged in tiers as shown in longitudinal sections. They taper abruptly at the ends and are about 150 μ in length, those of the rays being more nearly isodiametric. The zone of meristem between the secondary xylem and phloem is three to five cells wide. Like the cambial cells, the sieve tubes

and companion cells of the secondary phloem are arranged in tiers, while gliding growth is characteristic of cells differentiating as fibers.

Fibers in the root are confined to the phloem and secondary xylem, not occurring in the primary xylem, cortex, pericycle, or periderm. Those in the xylem often show distinct pits. They vary greatly in length, those measured being 0.6–3 mm. in length at maturity.

The earliest formed secondary xylem consists of a few vessels with long slender pits and very large diameter, scattered within compact tissue composed mostly of fibers or small tracheids, and a few parenchymatous cells grouped mainly around the vessels. Many of the parenchymatous cells later develop thick pitted walls distinguishing the earlier secondary growth from that occurring later.

The portion developed after the second month has somewhat less compact xylem (figs. 10, 11). Cells of the cambium differentiate as new rays; the primary rays widen until they are, by the fourth month, six or more cells in thickness near the cambium. There is a tendency for cambium initials to differentiate as fibers for some time, and then for a period to differentiate as vessels and tracheids in close succession, so that alternating layers of fibers and conducting tissue resemble rings of growth. No striking contrast, however, distinguishes the growth of the first season from that of the second in most of the root. This is not true in the hypocotyl and uppermost part of the tap root. In these, during the winter months, more cambium cells remain parenchymatous and the vessels formed are small. In winter and early spring of the second season large vessels and numerous tracheids are again formed, giving a contrast which serves, for a while at least, to demark the two seasons of the vegetative cycle. JONES (**6**), working with alfalfa, reports layers of crushed phloem in addition, indicating successive years' growth. This was not seen in *Melilotus*.

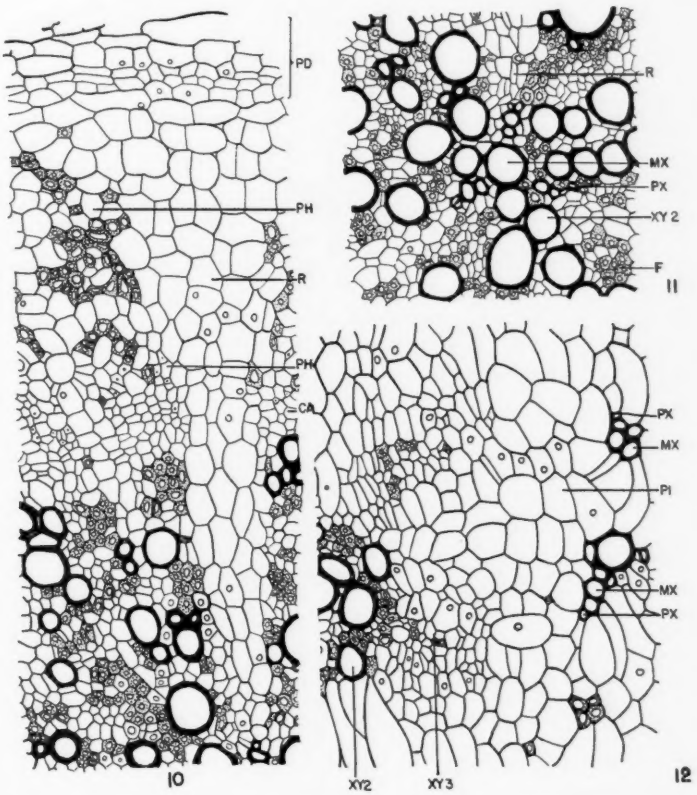
During the autumn and first season the most striking feature of secondary growth is the development of storage tissue. In this connection it will be necessary to consider the hypocotyl.

HYPOCOTYL AND DEVELOPMENT OF STORAGE TISSUE

In their study of the transition region in *Melilotus alba*, MCMURRY and FISK (**10**) describe the change from the typical triarch condition of the primary root to diarchy or tetrarchy in the lower hypocotyl and the disappearance of the exarch ridge of xylem on the intercotyledonary axis at higher levels, so that there is no direct connection with the primary xylem of cotyledonary or plumular traces. They mention also the differentiation of a conspicuous pith in the hypocotyl of the seedling.

Secondary growth from a vascular cambium proceeds in the hypocotyl in a manner similar to that in the root. Likewise a cork cambium develops in the endo-

dermis continuous with that of the upper part of the tap root, and at the same time. It forms, however, a conspicuous layer of secondary tissue before loss of the primary cortex and epidermis, which occurs after the hypocotyl is completely underground. The hypocotyl is thenceforth not easily distinguishable externally

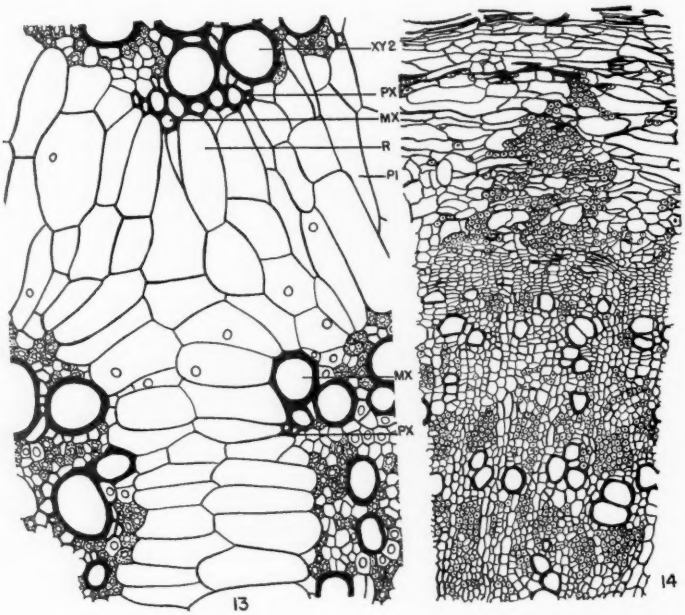


FIGS. 10–12.—Tap root of older plant: Fig. 10, transection of portion of tap root in June of first year showing secondary tissues. Fig. 11, same showing primary and secondary structures of central portion of stele. Fig. 12, central portion of tap root of second year showing tertiary tissue and separation of primary xylem elements by growth of parenchyma cells.

from the tap root. Internally the arrangement of the primary xylem makes such distinction possible until rapid growth of storage tissue involves the central elements of the stele.

In the tap root and hypocotyl, enlargement and radial divisions of cells in the pericycle and between the phloem groups become conspicuous in about the fourth

month, followed during the summer by general proliferation of the parenchyma, there and in the phloem itself. Phloem fibers become separated singly or in groups, and follow a sinuous course. During the same months all the rays are widened by multiplication and enlargement of their cells, but especially the central portions of the primary rays. Parenchyma associated with the metaxylem becomes active, the cells enlarge greatly, elongate tangentially, divide transversely, and then enlarge again. This results in separation of the metaxylem elements and in crushing



FIGS. 13, 14.—Tap root of plants of second year: Fig. 13, transection of central portion showing separation of elements of primary wood by growth of parenchyma. Fig. 14, transection of portion of dead root showing crushed phloem and thickened walls of parenchyma cells throughout most of area.

of the protoxylem (figs. 12, 13). The hypocotyl, upper part of the tap root, and larger secondary roots thus appear to possess a pith. Parenchymatous cells laid down close to the vessels of the secondary xylem, hitherto inconspicuous, enlarge and force the vessels and fiber groups apart. The activity of these cells in the central region of the hypocotyl is especially striking. The accumulation of starch in the storage tissue during the first season and its decrease during the second season have been described by WILLARD (**15**). Increase of the storage tissue continues in the second season but at a slower rate. A small amount of tertiary thickening may

occur in the pith, the cells maturing as fibers when any differentiation occurs (fig. 13).

What is apparently pith, which develops in the hypocotyl by proliferation of ray and xylem parenchyma, is to be distinguished in origin from the pith which first appears in the seedling associated with the primary tissues above the divergence of the two cotyledonary traces. In the old hypocotyl that pith appearing in the seedling is still conspicuous in the region of the cotyledonary node and is continuous with the pith of the stem rather than with that of the lower part of the hypocotyl, from which it is separated by a transverse plate of lignified tissue in which the parenchyma at the center of the stele becomes thick walled. This lignified tissue is confined to the cotyledonary node, suggesting that the hypocotyl in its structure is more nearly similar to the root than to the stem. It is readily distinguishable from the stem late in ontogeny as well as in the seedling.

Longitudinal sections through the old hypocotyl, at the time of flowering in the second season, show the primary vascular tissue and older vessels—and fibers of the secondary xylem in the central part of the stele—to be pushed laterally into sinuous folds by proliferating parenchyma, whereas the more recently formed vessels and fibers follow a straight course. This strongly suggests that proliferation of the parenchyma may have played an important role in the shortening and burial of the hypocotyl long before the cessation of secondary growth. JONES (**6**) suggests this explanation for shortening of the hypocotyl in alfalfa.

ROOT NODULE

The literature of the root nodule in the Leguminosae has recently been reviewed by FRED, BALDWIN, and MCCOY (**3**) and WILSON (**16**).

On the primary root of field-grown seedlings of *Melilotus* subjected only to chance infection by root nodule bacteria, nodules appear externally 7 days after germination of the seeds. At this age the unifoliate leaf has appeared but has not yet expanded. Even earlier than this nodules can be seen within the cortex of seedlings which have been cleared in chloral hydrate or in xylol. They become conspicuously enlarged and are more abundant by the tenth day, at which time the unifoliate leaf of the seedling is fully expanded. The fact that few nodules are formed before the appearance of the first true leaf in *Medicago* has been noted and regarded as significant by THORNTON (**12**). Secondary roots, after the appearance of root hairs, may also develop nodules.

Since divisions of the cells in the very young nodule are in many planes, a globular mass of tissue develops before the emergence of the nodule beyond the surface of the root. This enlarges and, after emergence, develops a cylindrical shape and becomes pink in color.

Transverse sections of mature nodules show the usual central mass of paren-

chymatous cells, most of which contain bacteria. A sheath of cells, containing starch in abundance, envelopes the central mass except at its distal end where a terminal meristem continually forms additional nodular tissue. The mature nodule has an outer cortical region of uninfected cells, beneath which—and outside the starch sheath—the nodule is traversed by two vascular strands differentiated back to the stele of the root opposite one, or occasionally two, of the protoxylem ridges.

The nodules of *Melilotus alba* are annual. Before the end of their growth they may become branched, the two vascular strands within the nodule likewise being branched repeatedly. At the end of the season the most central tissue of the nodule, containing the bacteria, disintegrate, leaving for a while a soft brown shell of cortical and vascular tissues. Eventually this too is lost and the bacteria within are returned to the soil.

A description of the ontogeny of the nodule is not undertaken here. Recent investigations of the possible role of growth substances, of metabolic products of the bacteria, and of growth-stimulating materials of the soil in the infection of roots and in the initiation and development of root nodules in the Leguminosae, and speculations in regard to these, have raised new questions and reopened old ones. Many of these have been pointed out by WILSON (**16**). Likewise cytological studies in which the tetraploid nature of the infected cells of the nodule has been described (**17, 18, 19**) seem to indicate that the whole matter of the ontogeny of the root nodule merits reinvestigation.

DEGENERATION AND DEATH OF ROOT

The period of flowering extends for about 6 weeks early in the summer of the second season. During this time the growth of the tops greatly exceeds that of the roots, and the weight of the root is reduced by loss of reserve foods (**15**). After subsequent formation of the last seeds the plant dies, usually in August or September. The root dies first.

Before death of the root, signs of degeneration appear. The older phloem is crushed by secondary growth, causing collapse of the parenchyma, sieve tubes, and some of the fibers. At the same time the interfascicular parenchyma collapses so that a sheath of dead tissue is formed beneath the periderm (fig. 14). Activity of the cambium ceases, as shown by the maturity of all the tissues most recently formed from it, so that the zone of undifferentiated cells between the xylem and phloem is narrowed down to a single layer of cells constituting the cambium. A marked increase in the number of fibers differentiated in the central part of the stele and general thickening of the walls of parenchymatous cells occur throughout the root, with the exception of the isolated periderm. Slight thickening of the walls of the youngest phloem and of the cambium cells takes place, occurring first in the rays, beginning near the center of the root, but becoming most marked in

the second year's growth. These changes occur first in the younger roots, progress to the older ones, and finally involve the tap root and hypocotyl.

The earliest indication of death of the tissues is shriveling of the cytoplasm. In sections of larger roots made before they seem to be dead, brown or black discolored areas occur. These mark the bases of dead lateral roots whose phloem gives the staining reactions characteristic of necrotic tissue. Through these lateral roots saprophytic fungi soon invade the larger roots, attacking the phloem, and then—following the rays inward—destroy the pith and finally plug the vessels by their growth. The entire life of the biennial plant occupies about 19 months from the time of germination of the seed.

Summary

1. The ontogeny of the primary root of *Melilotus alba* is of the fourth angiospermous type of JANCZEWSKI, in which there is a general meristem from which arises the cortex, stele, and central portion of the root cap. The meristem is extended laterally to form the outer parts of the root cap, and at higher levels a dermatogen.

2. Protophloem is the earliest stelar tissue differentiated.

3. Secondary roots arise by cell divisions in the pericycle opposite the protoxylem ridges. The endodermis forms a temporary root cap, which is ultimately crushed or digested, as are also cells of the cortex and epidermis of the primary root by the emerging root. The vascular system is developed in a manner similar to the primary root.

4. Lateral roots develop in the periderm and primary rays of old roots and of the hypocotyl. Cells of the periderm, by radial and tangential divisions, give rise to a temporary sheath over the young root, which is later ruptured. Subsequent development is similar to that of the secondary roots.

5. Secondary tissues develop from a cork cambium which arises in the endodermis, and from a vascular cambium which arises in the seedling between the xylem and phloem. A small amount of tertiary tissue may develop in the pithlike mass of the stele of the tap root late in ontogeny, maturing chiefly as fibers.

6. Parenchyma throughout most of the older roots, especially in the primary rays, becomes active late in the season, separating by its proliferation groups of vessels, fibers, and sieve tubes, crushing the protoxylem, and forming a pithlike mass. It serves as storage tissue for a large reserve of starch.

7. Withdrawal of the hypocotyl underground accompanies proliferation of the parenchymatous tissue and increase in the diameter of the hypocotyl and tap root late in the first season.

8. The hypocotyl has a pith above the level of divergence of cotyledonary traces continuous with that of the stem and present in the seedling. Late in on-

togeny it develops also a pithlike mass by proliferation of parenchyma in central parts of the stele, which is continuous with that of the root and homologous with it.

9. The root nodule originates in young roots near the close of primary differentiation. Two vascular strands are differentiated which are connected with the stele of the root opposite the nearest protoxylem ridges. An apical meristem adds cells to a central mass of infected and starch-bearing tissues and to a cortex which invests the entire nodule.

10. Root nodules are annual. New ones develop in succession on young roots.

11. Secondary growth continues in the second year through the period of flowering, but at a slower rate. Degeneration occurs after seeds are formed. The older phloem is crushed, the periderm becomes isolated by a layer of dead tissue beneath, most parenchymatous tissue throughout the root develops thick walls, and the cambium ceases to form new tissue. Increase of fibers in the center of the stele is marked.

12. The roots die before the tops; small roots die before the large ones. The tissues finally become disintegrated by the action of saprophytic fungi. Nineteen months is the length of life of the plant from the time of germination of the seed.

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EFFECT OF LENGTH OF INDUCTION PERIOD ON FLORAL DEVELOPMENT OF XANTHIUM PENNSYLVANICUM

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 528

FRANCES LLOYD NAYLOR

(WITH FOURTEEN FIGURES)

Introduction

Xanthium pennsylvanicum has been classified as a short-day plant. It tends to produce only vegetative growth on 24-hour cycles consisting of more than 16 hours of light and less than 8 hours of darkness and tends to flower when the photoperiods are shorter than $15\frac{1}{2}$ hours with accompanying dark periods of more than $8\frac{1}{2}$ hours. If exposed to a few cyclic alternations of light and darkness favorable for flowering, a plant may subsequently flower even though transferred to cycles which with continuous treatment would tend to result in purely vegetative activity. HAMNER (3) has termed these cyclic alternations of light and darkness which tend to induce flowering "photoinductive cycles." With *Xanthium* the so-called short day would constitute a photoinductive cycle; but, as he has shown, other cycles may induce floral initiation even though such cycles could not properly be considered under the term short day. In *Xanthium* a photoinductive cycle contains a specific minimum photoperiod of definite minimum intensity followed by a continuous dark period of more than 9 hours (3).

NEIDLE (5) and HAMNER (3) have found that the rate and nature of the development of the inflorescences of *Xanthium* are affected by the environmental conditions subsequent to the induction period. The former studied the effects of nitrogen supply and the latter primarily the effects of various conditions of light during the postinductive period.

It has been shown (4) in *Xanthium* that one photoinductive cycle may result in subsequent initiation of floral primordia. Under such conditions the rate of development of these primordia into mature flowers and fruits may be very slow. Since treatment with more than one photoinductive cycle seems to affect the rate of floral development, and since it has been shown with Biloxi soybean (1) also that the rate of floral development and the number of flowers initiated are affected by the number of photoinductive cycles to which the plants are exposed, an anatomical study of the floral development in plants which had been exposed to various numbers of photoinductive cycles was made.

Procedure

The experiments reported here were conducted in the summer of 1939. *Xanthium* plants were planted on August 1 in sandy garden soil in clay pots and grown in the greenhouse. During the first 25 days all plants were grown under conditions of long photoperiod, being exposed to supplementary illumination from dusk until midnight. This illumination was obtained from 200-watt Mazda filament lamps and supplied about 80 foot candles at the surface of the leaves. Subsequent to the twenty-fifth day the plants were exposed to the various experimental treatments. In these treatments continuous illumination was obtained by burning the lamps from dusk until sunrise. Photoinductive cycles (short photoperiods) were produced by placing the plants on trucks and moving them into dark compartments at 4:00 P.M., where they remained until 8:00 A.M. the following morning. From then until 4:00 P.M. the trucks stood in the lighted greenhouse.

On the twenty-fifth day the terminal buds of some of the plants were removed and preserved for subsequent examination. All such buds proved to be strictly vegetative, and the assumption is made that all comparable plants were vegetative at this time. As a further check, certain of the plants were continued for 4 more days under long-photoperiod conditions on the greenhouse bench, and samples were preserved and examined histologically. These were also strictly vegetative. The remaining plants were started on the various experimental treatments after the twenty-fifth day (August 25). One lot of 320 plants was exposed continuously to photoinductive cycles, and terminal buds of ten plants were collected each day for 32 days and preserved for histological examination. These plants of lot 1 were used as a basis of comparison. The second lot, containing 50 plants, was transferred to continuous illumination; the third lot of 100 plants was exposed to one photoinductive cycle; a fourth lot of 100 plants, to four photoinductive cycles; and a fifth lot of 100 plants, to eight photoinductive cycles. Lots 3–5, inclusive, were placed under continuous illumination subsequent to their photoinductive treatment. The terminal nodes of ten plants of each of lots 2, 3, 4, and 5 were collected at weekly intervals for 10 weeks and preserved for histological examination, the first collection being made September 2.

The specimens were preserved in formalin-acetic-alcohol and handled according to the butyl-alcohol paraffin method. Serial longitudinal and transverse sections were cut at 15 μ and stained with safranin, gentian violet, and orange G.

Observations

Control plants

The terminal buds of the plants collected on August 25 were strictly vegetative, as were those from the same bench collected on August 29. The plants of lot 2, which were transferred on August 25 to continuous illumination, were all typically

vegetative at each sampling (fig. 1). From these results it is assumed that all plants were vegetative on August 25, and that plants of groups 3, 4, and 5 which developed floral primordia and flowers while growing in continuous illumination did so because of previous photoinductive treatment.

Continuous photoinductive treatment (lot 1)

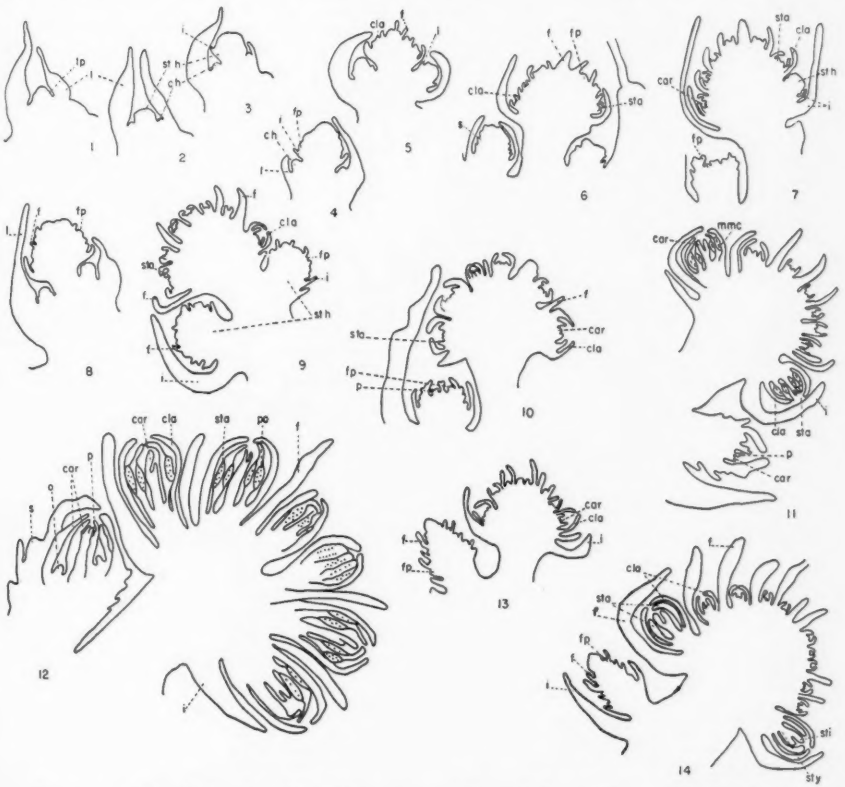
The terminal buds of plants collected on the first and second days after the beginning of photoinductive treatments showed no noticeable change from the vegetative condition, but on the third day the terminal growing point was broad, smooth, somewhat elevated, and hemispherical (fig. 2). This was the primordium of the terminal staminate inflorescence. By the fourth day the primordia of the involucral bracts appeared at the base of the terminal staminate inflorescence. In the axils of the young leaves, just below the staminate inflorescence, there appeared the primordia of the carpellate inflorescences (fig. 3). By the fifth day the first flower primordia of the staminate inflorescence were noticeable and the carpellate inflorescence primordia had enlarged to about 0.15 mm. (fig. 4). From this point on the development of the staminate and carpellate inflorescences is described separately.

Staminate inflorescence.—The flower primordia and the primordia of the floral bracts developed in spiral acropetalous succession in the staminate inflorescence and were numerous by the sixth day (fig. 5). The primordia of the central flowers had flattened out and the primordia of the marginal flowers had not only flattened but the initials of the corolla were present at their margins. By the seventh (fig. 6) and eighth days (fig. 7) the inflorescence was about 1.3 mm. in diameter, and the slender peduncle by which the terminal staminate inflorescence is raised above the carpellate inflorescences had elongated, its length being about equal to the diameter of the staminate inflorescence. The primordia of the corolla were present in the central flowers, and in the marginal flowers the corolla primordia and also the stamen primordia were present, the latter arising adaxial to the corolla. Each flower was in the axil of a bract.

At this time one or two small staminate inflorescences (about 0.26 mm. in diameter) were noted within the terminal staminate inflorescence, each small one appearing in the axil of an involucral bract of the terminal inflorescence. Flower and floral bract primordia were present in the small infloresences.

On the ninth day (fig. 10) the terminal staminate inflorescence was 1.8 mm. in diameter. Each flower had a marginal sympetalous corolla, a column composed of five or six undiverged stamens, and the primordia of two undiverged carpels in the center. In some flowers a cleft had appeared at the apex of the carpel primordia. The calyx was absent. By the thirteenth day (fig. 12) each stamen was differentiated into filament and anther. Pollen was shed about the eighteenth day after the

beginning of induction. The rudimentary pistil of each staminate flower consisted of a style with a bifid stigma. The period during which pollen was shed extended beyond the time of shedding from the terminal inflorescence, since the flowers of



FIGS. 1–14.—Terminal region of *Xanthium* represented in each figure. Fig. 1, vegetative plant. Fig. 2, third-day stage of plant which had received continuous photoinductive treatment (lot 1). Fig. 3, fourth day of same. Fig. 4, fifth day. Fig. 5, sixth day. Fig. 6, seventh day. Fig. 7, eighth day. Fig. 8, eighth-day stage of plant which had received one photoinductive cycle followed by continuous illumination (lot 3). Fig. 9, eighth-day stage of plant which had received four photoinductive cycles. Fig. 10, ninth-day stage of lot 1. Fig. 11, eleventh-day stage of lot 1. Fig. 12, thirteenth day of same. Fig. 13, thirty-sixth-day stage of lot 3. Fig. 14, sixty-fourth-day stage of lot 3. (*car*, carpel; *cla*, corolla; *ch*, carpellate inflorescence; *f*, floral bracts; *fp*, flower primordium; *i*, involucral bract; *l*, leaf; *lp*, leaf primordium; *mmc*, microspore mother cell; *o*, ovule; *po*, pollen; *s*, spine; *sta*, stamens; *sti*, stigma; *sth*, staminate inflorescence; *p*, perianth; *sty*, style.)

the small inflorescences in the axils of the involucral bracts developed more slowly than did the flowers of the terminal inflorescence itself.

CARPELLATE INFLORESCENCE.—The primordia of the involucral bracts were

present at the base of the primordium of the topmost carpellate inflorescence by the sixth day (fig. 5). By the seventh day the inflorescence was 0.6 mm. in diameter and many protuberances had appeared on the surface. The lower ones, about fifteen in all, were the primordia of the involucral bracts; the upper ones were the primordia of the spines. The receptacle of the carpellate inflorescence was flattened at the apex (fig. 6). The margins of the receptacle, and the tissue at the exact center between the margins, became slightly elevated. Continued growth of these regions resulted in the formation of two depressions, which became wide at the bottom and narrow above. On one side the margin grew more rapidly and thus one depression became deeper than the other.

By the eighth day flower primordia appeared simultaneously in the bottom of each depression. The flower in the lower depression developed somewhat more rapidly and became larger than the other. The central tissue, which was a partition between the two depressions, developed a cleft in its upper surface (fig. 7). By the ninth day (fig. 10) the primordium of the perianth was evident at the margin of each flower primordium. By the eleventh day the two carpels had appeared in the center of the flower (fig. 11). The upward growth of the carpels formed the single cavity of the ovary. The carpels grew much more rapidly than did the perianth, and by the thirteenth day (fig. 12) the perianth appeared as a small flange on the outer margin of the carpels. The carpels continued elongation and formed a long style and a bifid stigma. About the twelfth day an ovule began developing in each ovary.

All flower parts were present by the thirteenth day, and further development consisted of increase in length and breadth. The megagametophyte was mature by the seventeenth day. The margin and central tissue of the receptacle grew far above the flowers. One part of the central tissue, with half the margin, formed the beak above one flower. The other half of the central tissue and the other half of the margin formed the beak above the second flower.

The carpellate inflorescences in the axils of the third and fourth leaves below the terminal staminate inflorescence developed more rapidly and became larger than did the uppermost carpellate inflorescences. For example, on the eighth day the flower primordia were present in the depressions of the uppermost inflorescences, but in the lower inflorescences the perianth and carpel primordia were already differentiated. At the end of a month the seed of the uppermost bur was about 3.5 mm. long and the embryo about 1.5 mm. long. The lower bur contained a seed 6.5 mm. long and an embryo 2.5 mm. long.

FARR (2) has stated that the bur is a modified capitulum and that it differs from the typical capitulum of the Compositae only in having two depressions in the receptacle. He believes that the spines are specialized bracts and the beaks are portions of the receptacle.

Various inductive treatments (lots 3–5)

1. One photoinductive cycle.—The inflorescences and flowers of these plants were greatly delayed in development as compared with those which received continuous photoinductive treatment (lot 1). By the eighth day (fig. 8) after induction the staminate inflorescence showed the primordia of the flowers and leaf bracts, but no flower parts had differentiated. The primordia of the involucral bracts of the inflorescences in the axils of the young leaves were just visible. This stage was shown on the fifth day by the plants of lot 1. By the fifteenth day the marginal flower primordia of the terminal staminate inflorescence showed flattened apices. The floral bracts were clearly distinguishable from the flower primordia, a stage comparable with that of the plants of lot 1 on the sixth day. The axillary inflorescences showed little advance in development, the inflorescences being somewhat cone-shaped, the involucral bracts differentiated, but the type of inflorescence not yet distinguishable.

By the twenty-second day the marginal flowers of the terminal staminate inflorescence had developed the primordium of the corolla and a few of the central flowers had flattened apices, a stage comparable with the plants of lot 1 on the seventh day. The axillary inflorescences showed no change in development. By the twenty-ninth day the marginal flowers of the terminal staminate inflorescence resembled those of lot 1 on the eighth day, having the corolla, stamen, and carpel primordia established; but the most central flowers were just beginning to show corolla primordia. There was a greater difference in the size and stage of development between the outer and inner flowers of this inflorescence than in those of lot 1. This difference became increasingly marked as the plants grew older. The peduncle by which the terminal staminate inflorescence is raised above the topmost leaves was longer than in the plants of lot 1.

In the terminal staminate inflorescence there were one or two axes, each appearing in the axil of an involucral bract of the terminal staminate inflorescence. Each axis was composed of one small terminal and two small lateral staminate inflorescences, rather than of a single staminate inflorescence as in lot 1. These small inflorescences had flower primordia and bracts but no flower parts differentiated. Unlike the small staminate inflorescences of lot 1, these were elongated. The internodes of each small inflorescence had continued longitudinal growth and the individual flowers developed one above the other on the axis in the axils of the floral bracts. By the thirty-sixth day the small staminate inflorescences had increased in size, and by the forty-third day the floral bracts were very long. It seems probable that these floral bracts may become the leafy organs often noted on staminate inflorescences of *Xanthium* plants which have received a few induction periods and then been grown on long photoperiod for several months (**5**).

An individual carpellate inflorescence or an axis consisting of three carpellate

inflorescences usually appeared in the axils of the first, second, third, and fourth leaves below the terminal staminate inflorescence in the plants of lot 1. In some of the plants which received only one photoinductive cycle (lot 3) a lateral axis consisting of three small staminate inflorescences appeared in the axil of the topmost leaf. These inflorescences were elongated rather than round. In the terminal inflorescence of this lateral axis flower primordia and floral bracts were present. In the axils of other upper leaves there were carpellate inflorescences. In the axils of leaves below these the primordia were so slightly developed that their type could not be determined.

By the thirty-sixth day all the flower parts had formed in the marginal flowers in the terminal staminate inflorescence, the largest staminate inflorescence of the plant. The central flowers, however, were still in the primordial stage (fig. 13). By the sixty-fourth day nearly all the central flowers of the terminal staminate inflorescence had formed corolla and stamen primordia (fig. 14). Carpellate inflorescences in which the depressions were beginning to form were found in the axils of some of the upper leaves of the plant. The inflorescences did not develop further before the end of the experimental period.

2. FOUR PHOTOINDUCTIVE CYCLES.—These plants had received four photoinductive cycles (four short photoperiods) and 4 days of continuous illumination when the first samples were taken at the end of the eighth day (fig. 9). In the terminal staminate inflorescence the individual flowers were nearly as well developed as were those of lot 1. The one or two small staminate inflorescences which appeared in the axils of involucral bracts of the terminal staminate inflorescence were about three times as large as those on the plants of lot 1, being about 0.8 mm. in diameter. Their flower and bract primordia could be distinguished, but no flower parts had been differentiated. The staminate inflorescences were round, not elongated like the small ones on the plants which had received one cycle of induction. Subsequent development was somewhat delayed, a stage comparable with that of lot 1 on the eleventh day being reached by the fifteenth day. The first pollen was shed about 3 weeks after the shedding by plants of lot 1. The megagametophytes were mature by the thirty-sixth day. The inflorescences in the axils of the involucral bracts formed their flower parts later than did the terminal inflorescence itself.

3. EIGHT PHOTOINDUCTIVE CYCLES.—At the time of the first collection of lot 5, 8 days after the beginning of the photoinductive treatments, these plants had not yet been moved to continuous illumination. They were in the same stage of development as were the plants of lot 1 on the eighth day. Samples from later collections, taken after these plants had been moved to continuous illumination, showed that development (as compared with lot 1) was only slightly delayed. By the twenty-second day the megagametophytes were mature. Pollen was shed about 5 days after the shedding by the plants of lot 1.

The number of staminate and carpellate inflorescences in the upper five nodes of

plants which had received one, four, and eight photoinductive cycles was compared with the number on the plants under continuous photoinductive treatment (lot 1). Stages from each group, comparable with the thirteenth day of the latter (the eighty-fourth-day stage of the plants induced one day, etc.), were selected because at this stage the character of the inflorescences could best be determined.

On the plants of lot 1 there were present a terminal staminate inflorescence and also two small staminate inflorescences, each in the axil of an involucral bract of the terminal inflorescence. The inflorescences in the axils of the four topmost leaves were carpellate. In some plants the axis in the axil of the fifth leaf below the terminal staminate inflorescence bore lateral carpellate inflorescences and terminated in a staminate inflorescence. There were three or four staminate and twelve or thirteen carpellate inflorescences per plant at the upper five nodes.

On the plants which received one photoinductive cycle the main axis terminated in a staminate inflorescence. Two lateral axes, each in the axil of an involucral bract of the terminal inflorescence, were each composed of three small staminate inflorescences. In the axil of the topmost leaf of some plants there were three staminate inflorescences; those in the axils of two other upper leaves were carpellate. In many plants the axes in the axils of the fourth and fifth leaves below the terminal staminate inflorescence were so slightly differentiated that it was impossible to tell whether they were vegetative or floral. There were about ten staminate and six carpellate inflorescences in the upper five nodes.

The plants which received four photoinductive cycles and the ones which received eight had about the same number of staminate and carpellate inflorescences as had the plants which received continuous photoinductive cycles (lot 1).

Summary

1. Histological examinations were made of the developing carpellate and staminate inflorescences of *Xanthium pennsylvanicum*. The plants were exposed after the twenty-fifth day of vegetative growth to (*a*) continuous photoinductive cycles, (*b*) one photoinductive cycle, (*c*) four photoinductive cycles, (*d*) eight photoinductive cycles. The study included those inflorescences which developed from the terminal bud and the buds in the axils of the five uppermost leaves.

2. Development of both carpellate and staminate inflorescences in the plants exposed continuously to photoinductive cycles was more rapid than that in any other group. All flower parts were present by the thirteenth day, and at the end of the month the seeds were almost mature.

3. Development of inflorescences in the plants which received only one photoinductive cycle was much slower than that of plants which received continuous photoinductive treatment. The flower parts of the former did not appear in all the flowers of the terminal staminate inflorescence until the sixty-fourth day after induction, rather than by the thirteenth day as in the latter. The former also dif-

fered from the latter in that there was a greater difference in the size and stage of development between the outer and inner flowers of the inflorescence.

4. The terminal staminate inflorescence of the plants receiving one photoinductive cycle, as well as of the plants exposed continuously to photoinductive treatment, contained one or two axes, each appearing in the axil of an involucral bract of the terminal inflorescence. Each axis of the former was composed of three staminate inflorescences, rather than of a single one as in the latter. The small staminate inflorescences of the former were much more elongated than those of the latter.

5. In the axil of the uppermost leaf a lateral axis consisting of three staminate inflorescences appeared in some of the plants exposed to one photoinductive cycle, while in those exposed continuously to photoinductive treatment an individual carpellate inflorescence or an axis consisting of three usually occupied this position. The staminate inflorescences of the former were elongated rather than round.

6. In the plants exposed to one photoinductive cycle there developed approximately ten staminate and six carpellate inflorescences, while in the plants exposed continuously to photoinductive cycles there was an average of about four staminate and twelve or thirteen carpellate inflorescences per plant. Thus, increasing the number of induction treatments seemed to stimulate the production of carpellate more than of staminate inflorescences.

7. The plants which received four photoinductive cycles exhibited, in the axils of involucral bracts of the terminal staminate inflorescence, one or two staminate inflorescences three times as large as those occupying this position in plants exposed continuously to photoinductive treatment.

8. The development of the inflorescences on those plants which received four or eight photoinductive cycles resembled more closely the development in plants continuously exposed to such treatment than that in plants exposed to one photoinductive cycle. The rate of development was intermediate between the two.

The writer expresses her appreciation of the helpful suggestions given by Dr. Karl C. Hamner during the course of these experiments.

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COMBINED EFFECTS OF POTASSIUM SUPPLY AND GROWTH SUBSTANCES ON PLANT DEVELOPMENT[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 529

HORTON M. LAUDE

(WITH SIX FIGURES)

Introduction

The reported dry-weight determinations of plants treated with growth-regulating substances have indicated both losses and gains associated with such treatment. GRACE (4), HWANG and PEARSE (6), MCROSTIE, HOPKINS, and GRACE (8), MITCHELL and HAMNER (9), and THIMANN and LANE (17) have all reported increased dry weight of various plants owing to such treatments. PEARSE (12, 13) found the dry weight of leaf and root decreased while that of stem and petiole increased in tomatoes, yet the total dry weight did not increase. TEMPLEMAN (16), using white mustard and barley, reported only significant depressing effects from growth substance treatment and no stimulation of top or root weights.

The combined effects of variation in nutrient supply and growth-regulating substances have been studied in a few instances. Nutrition as it affects the production of growth substance within the plant has been investigated by AVERY, BURKHOLDER, and CREIGHTON (1, 2) and by SKOOG (15). Nutritional levels and growth-substance treatments in relation to root development and carbohydrate and nitrogen distribution within the plant have been reported by SMITH, NASH, and DAVIS (14). EATON (3) suggested the ability of externally-applied indoleacetic acid partially to replace boron as an essential element. SKOOG (15) noted that applied indoleacetic acid may enhance the utilization of zinc.

The investigation here reported deals with the effects—at different levels of potassium—of growth substance supplied in the nutrient medium to sand cultures of kidney bean.

Material and methods

Red kidney bean, *Phaseolus vulgaris*, was grown in white quartz sand in 4-inch glazed pots. Before planting the seed the sand was thoroughly wet with distilled water and given one application of minus potassium nutrient. This first addition of nutrient was necessary to insure uniform and vigorous plants soon after emer-

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gence; without it the plants did not properly expand their first foliage leaves. Four selected seeds were planted at uniform depth in each pot. As soon as the heart-shaped leaves were expanded, the plants were thinned to two per pot. Seed coats and ungerminated seeds were removed.

Five nutrient solutions, designated as minus K, 1/18 K, 3/9 K, 9/9 K, and 18/9 K, were prepared in the following manner. The amount of 0.5M stock solution of the reagent quality salts used for every liter of nutrient solution is listed. Distilled water was employed in all solutions.

The minus K nutrient solution consisted of 12 cc. of 0.5M $Ca(NO_3)_2$, 9 cc. of 0.5M $MgSO_4$, and 9 cc. of 0.5M NaH_2PO_4 per liter. The 1/18 K nutrient solution contained 12 cc. of 0.5M $Ca(NO_3)_2$, 9 cc. of 0.5M $MgSO_4$, 0.5 cc. of 0.5M KH_2PO_4, and 8.5 cc. of 0.5M NaH_2PO_4 per liter. The 3/9 K nutrient solution consisted of 12 cc. of 0.5M $Ca(NO_3)_2$, 9 cc. of 0.5M $MgSO_4$, 3 cc. of 0.5M KH_2PO_4, and 6 cc. of 0.5M NaH_2PO_4 per liter. The 9/9 K nutrient solution employed 12 cc. of 0.5M $Ca(NO_3)_2$, 9 cc. of 0.5M $MgSO_4$, and 9 cc. of 0.5M KH_2PO_4 per liter. The 18/9 K nutrient solution consisted of 12 cc. of 0.5M $Ca(NO_3)_2$, 9 cc. of 0.5M $MgSO_4$, 9 cc. of 0.5M KH_2PO_4, and 9 cc. of 0.5M KCl per liter. A somewhat higher osmotic concentration resulted in the 18/9 K level owing to the addition of KCl to the other salts. All nutrient solutions uniformly contained iron, boron, manganese, and zinc at 0.5 p.p.m. and copper at 0.125 p.p.m.

Indoleacetic acid (Merck and Company) and alpha naphthalene acetamide (American Chemical Paint Company) were employed as the growth substances. They were used individually in series of concentrations ranging from 10^{-4} to 10^{-11} by multiples of ten at the several levels of nutrition. Fresh stock solutions of the growth substances were prepared for each watering. The proper amount of these solutions was mixed with the nutrient to give the desired concentration immediately before watering the plants. Applications were given three times a week between the first treatment, 10 days after planting, and harvest at the flowering stage. The volume of nutrient solution was increased as the plants matured, so that there was always a slight run off through the pot drain after each application.

Fresh and dry weights were taken for the tops, and dry weights for the roots. The roots were freed of sand by washing over a screen, dipping in saturated salt water, and rinsing in clear water. The material was dried for 24 hours in a well-ventilated draft oven at 80° C. Samples removed from the oven were kept in desiccators until weighed.

The experiments were designed on a factorial system, with treatments randomized within blocks; and an analysis of variance (11) was used to establish the significant points regarding top dry weight, root dry weight, and total plant dry weight.

Investigation

Indoleacetic acid

Experiment I.—This ran from October 10 to November 7, 1940. During this period 60 per cent of the days were sunny and growing conditions were good. Plants reached the flowering stage in 28 days after planting the seed. Nutrient levels of minus K, 1/18 K, 3/9 K, and 9/9 K were used. An analysis of variance of the dry weight of the tops indicated significant increases at indoleacetic-acid concentrations near 10^{-9} over plants receiving the same 9/9 K nutrient but without the growth substance. The differences in response between plants receiving and those not receiving the acid at the 3/9 K, 1/18 K, and minus K levels were not significant. There was a tendency for dry weight of roots to increase with increasing concentrations of the growth substance and for dry weight of the tops to decrease at the higher concentrations. Also a definite decrease in succulence was associated with decreasing amounts of potassium.

Experiment II.—A more extensive experiment was designed having six blocks, within each of which thirty-one treatments were randomized (table 1). There were eight plants to a treatment in each block, and thus forty-eight plants to a treatment in the entire experiment. The results of this experiment illustrate those of the first as well.

This experiment was carried on between January 24 and February 25, 1941. Only 40 per cent of the days were sunny, there being less light than in the autumn. Thirty-two days were required for flowering. During this time ten applications of nutrient and growth substance were given. The greenhouse temperature averaged 70° F., and the average relative humidity ranged from 60 to 70 per cent.

No epinastic response was noted, even at the 10^{-4} and 10^{-5} concentrations after applications. This was in contrast to phosphorus-deficient and boron-deficient bean plants grown at the same time and receiving the same indoleacetic-acid concentrations, for these showed definite epinastic curvatures of the primary leaves. The first trifoliate leaves of the plants at the 10^{-4} concentration were very pale green compared with the 10^{-5} or any other treatment. This partial chlorosis persisted in the second and third trifoliate leaves as they appeared. Likewise the first obvious signs of stunting appeared at this concentration. By the eighteenth day after planting (twelfth after emergence), height differences were marked enough to distinguish clearly between the taller plants of the upper two potassium levels and the shorter plants of the lower two.

The first visible symptom of potassium deficiency appeared on the nineteenth day after planting in the minus K treatments. It required 6 additional days for this condition to become obvious in the 1/18 K plants. This first symptom was shown by the action of the pulvinus at the base of the primary leaf blade. Those

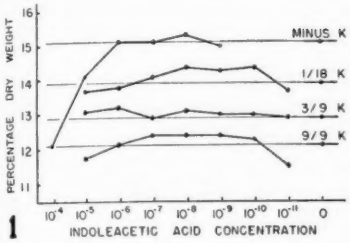
leaf blades which would a short time later exhibit the characteristic marginal chlorosis of deficiency would retain the nocturnal pendant position during the day. All others remained horizontal except at night. The early marginal yellowing of the deficient leaf blades was followed by more extensive chlorosis, until the entire leaf blade was chlorotic. The veins were the last portion so affected. By the time of harvest the primary leaves had begun to abscise and the first and even second trifoliate leaves were somewhat mottled with chlorotic areas.

By the twenty-seventh day after planting, flower buds were evident on all plants regardless of treatment. At harvest on the thirty-second day a few of these buds had commenced to open. Leaf size varied with concentration of growth substance supplied. The more stunted plants at the higher concentrations had considerably less leaf area than had those plants receiving a more favorable concentration. All plants, regardless of treatment, had at the same nutrition level the same total number of leaves.

The percentage dry weights for the bean tops are shown in figure 1. Although the range of the percentages is only slightly over 3.5 per cent, there does seem a clear-cut response to each nutritional level, and this response in a definite order. Succulence increases with increasing amounts of potassium. With the exception of the two highest concentrations of indoleacetic acid at minus K, all the points are well stratified. There seems little relationship between quantity of growth substance and percentage dry weight over the central range of the concentrations used. However, the rapid drop in percentage dry weight with the greatest amount of indoleacetic acid suggests that high concentrations may have a more measurable effect. Increases in succulence as a result of supplied growth substance have been reported, and it appears likely that they were associated with relatively high concentrations.

The dry-weight data of total plant, top, and root represent average weights for each treatment (table 1). An analysis of variance to establish the differences between these means necessary for significance at probabilities of 5 per cent and 1 per cent gave the following results. Mean differences are significant if greater than 1.00 gm. at 5 per cent and 1.32 gm. at 1 per cent probability for dry weight of total plant; 0.47 gm. at 5 per cent and 0.62 gm. at 1 per cent probability for dry weight of tops; and 0.22 gm. at 5 per cent and 0.29 gm. at 1 per cent probability for dry weight of roots.

A comparison of the nutrition levels, aside from the effect of growth substance, may be made from the controls where no indoleacetic acid was used. The dry weights for the roots indicate no significant differences among the four levels. In regard to dry weight of total plant and tops, however, these four levels differ significantly at 1 per cent probability in all comparisons except that of the 3/9 K with the 9/9 K level. These two are nearly alike in actual weights. During growth



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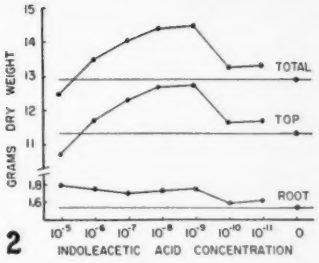

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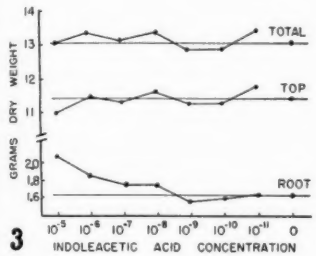

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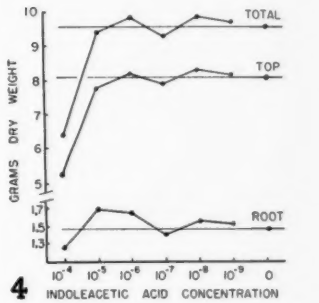

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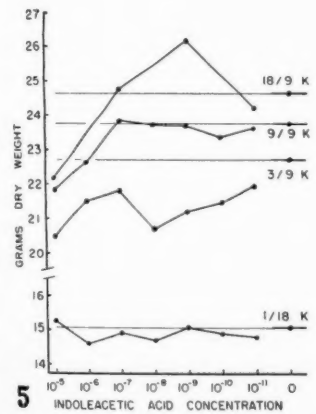

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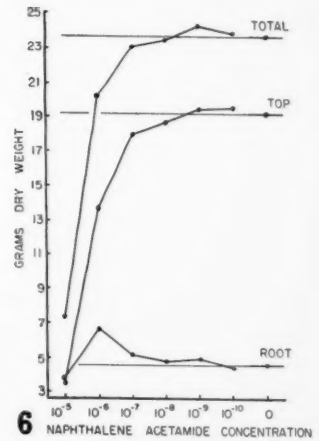

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FIGS. 1–6.—Each straight horizontal line represents the value for the specific control treatment which received no growth substance, for comparison with the lots which did. Fig. 1, percentage dry weight of bean tops at four levels of potassium. Fig. 2, dry weights for total plant, top, and root, at 9/9 K level (experiment II). Fig. 3, dry weights for 3/9 K level (experiment II). Fig. 4, dry weights for minus K level (experiment II). Fig. 5, dry weights of total plant at four potassium levels of experiment III. Fig. 6, dry weights for 9/9 K level of experiment IV.

both appeared much the same. Neither showed potassium deficiency symptoms nor any differentiating peculiarities. If based on the control-treatment results, the minus K, 1/18 K, and combined upper two levels seem to constitute the only distinct conditions. Yet a marked difference appears between the 9/9 K and 3/9 K levels in their response to the indoleacetic-acid concentration series (table 1; figs. 2, 3). At 10^{-9} the 9/9 K level yields 12.5 per cent increase in total dry weight over the control at that level, while the same concentration of growth substance with the 3/9 K level is slightly below its corresponding control. The 9/9 K level has a definite curve of response, with 10^{-7}, 10^{-8}, and 10^{-9} concentrations being significant increases and the latter two at 1 per cent probability, while the trend of

TABLE 1

DRY WEIGHT OF TOTAL PLANT, TOP, AND ROOT. FIGURES REPRESENT MEAN WEIGHT IN GRAMS OF SIX SAMPLES OF EIGHT PLANTS EACH

Nutrition level and plant part		Indoleacetic-acid concentration								Control (no acid)
		10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	
9/9 K	Total plant	.....	12.50	13.50	14.03*	14.44†	14.49†	13.27	13.35	12.88
	Top	.....	10.70†	11.74	12.32†	12.70†	12.73†	11.68	11.72	11.35
	Root	.....	1.79*	1.76*	1.71	1.74	1.76*	1.59	1.62	1.53
3/9 K	Total plant	.....	13.00	13.26	13.07	13.28	12.76	12.81	13.32	12.99
	Top	.....	10.94	11.40	11.32	11.53	11.21	11.23	11.68	11.35
	Root	.....	2.07†	1.86	1.75	1.75	1.55	1.59	1.64	1.64
1/18 K	Total plant	.....	10.92	10.94	11.91	11.96	11.22	11.63	11.15	11.34
	Top	.....	9.18*	9.29	10.25*	10.16	9.60	9.93	9.58	9.70
	Root	.....	1.74	1.64	1.66	1.79	1.62	1.70	1.57	1.64
Minus K	Total plant	6.48†	9.42	9.80	9.28	9.86	9.69	.....	.....	9.57
	Top	5.24†	7.71	8.14	7.87	8.30	8.14	.....	.....	8.09
	Root	1.24*	1.70	1.66	1.41	1.56	1.55	.....	.....	1.48

* Significant difference at 5 per cent probability from control at same nutrition level.
† Significant difference at 1 per cent probability from control at same nutrition level.

the 3/9 K level is irregular with no significant points. In this case plants appearing alike but receiving different nutrition responded in a decidedly different manner to indoleacetic-acid applications.

Much the same reaction occurs in relation to dry weight of tops. At 9/9 K significant increases are found at concentrations of 10^{-7}, 10^{-8}, and 10^{-9}, while the 10^{-5} concentration is depressed relative to the control. In the 3/9 K level no point is significant, either above or below the control. Only when root weights are considered does a similarity of response appear. At 10^{-5} concentration, root weights are significantly increased at both levels. A trend for increased root weight with the higher concentrations of indoleacetic acid is indicated.

The 1/18 K and minus K levels exhibited decided potassium deficiency symptoms and differed only in their intensity. Few significant weight differences were obtained at these levels (table 1; fig. 4). The 1/18 K level has significant decrease

at 10^{-5} and increase at 10^{-7} for the tops. On the basis of total plant weight, however, these points are not significant. No definite trend in relation to the indoleacetic-acid concentrations appears at either level. This irregular response does not indicate that indoleacetic acid can replace potassium or lessen the effect of potassium deficiency, as judged by the response of the plant as a whole.

Significant decreases in all three weights at the 10^{-4} treatment provide quantitative evidence for the stunting effect of growth substance under conditions of high concentration. This depression of growth probably would have occurred at any of the nutrition levels with a similar high concentration.

EXPERIMENT III.—To obtain data under growing conditions of higher light intensity, an experiment designed like the one just described but with eight blocks instead of six, and twenty-nine treatments randomized within each block, was carried out between March 17 and April 17, 1941. In this one the minus K level and the 10^{-4} indoleacetic-acid concentration were omitted and an 18/9 K level included. Sixty per cent of the days were sunny, and at noon on clear days light intensity in the greenhouse reached 5500–6000 foot candles. This was indicated in the increased dry weights. For example, the total dry weight of the control plants at the 9/9 K level was 85 per cent greater in this experiment than in experiment II.

Procedure in the two experiments was identical. Ten applications of the various levels of nutrient and growth substance were given. The plants were harvested when the flower buds were well developed, 31 days after planting the seed. Again there were no epinastic curvatures following indoleacetic-acid applications. The pendant position during the day of the primary leaf blades of potassium-deficient plants was observed as the first symptom of such deficiency. Flower buds appeared at the same time on all treatments. The total number of leaves per plant was the same at each nutrition level, regardless of treatment. Noticeable differences were apparent in leaf size and length of stem and petiole. Percentage dry weight decreased with increasing potassium, and the 18/9 K level averaged about 1 per cent lower than the 9/9 K level.

One condition was observed which was not detected in experiment II. On the last days before harvest the primary leaves of many of the 3/9 K level plants showed definite symptoms of potassium deficiency. The environmental conditions resulted in increased growth which apparently brought out the deficiency at this level.

Dry weights of total plant, top, and root were analyzed as before (table 2). Mean differences are significant for dry weight of total plant if greater than 1.46 gm. at 5 per cent probability and 1.93 gm. at 1 per cent probability; for dry weight of top if greater than 0.91 gm. at 5 per cent probability and 1.20 gm. at 1 per cent probability; and for dry weight of root if greater than 0.62 gm. at 5 per cent probability and 0.82 gm. at 1 per cent probability.

Root weights indicate few important differences and no obvious trends relative

to growth substance treatment. The control at 1/18 K is markedly lower than the three higher levels, which cannot be separated among themselves.

Dry weights of tops parallel dry weights of total plant (fig. 5). Consideration of these dry weights of entire plants in the control treatments to observe the effect of the nutrition levels without the growth substance indicates three conditions. The 1/18 K level is significantly lower than the other three. By harvest it exhibited pronounced deficiency symptoms. The 3/9 K level is lower than the 18/9 K level but does not vary significantly from the 9/9 K level. The 3/9 K plants developed the early deficiency symptoms just before harvest. The 9/9 K and the 18/9 K levels cannot be separated on the basis of dry weight of controls.

TABLE 2

DRY WEIGHT OF TOTAL PLANT, TOP, AND ROOT. FIGURES REPRESENT MEAN WEIGHT IN GRAMS OF EIGHT SAMPLES OF EIGHT PLANTS EACH

NUTRITION LEVEL AND PLANT PART		INDOLEACETIC-ACID CONCENTRATION							CONTROL (NO ACID)
		10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	
18/9 K	Total plant	22.14†	.....	24.71	.....	26.13*	.....	24.23	24.62
	Top	18.17†	.....	20.47	.....	21.60*	.....	20.04	20.50
	Root	3.98	.....	4.24	.....	4.54	.....	4.20	4.12
9/9 K	Total plant	21.81†	22.64	23.83	23.70	23.68	23.34	23.58	23.79
	Top	17.84†	18.80*	19.97	20.05	19.88	19.73	19.70	19.84
	Root	3.97	3.85	3.87	3.66	3.80	3.62	3.87	3.95
3/9 K	Total plant	20.44†	21.50	21.80	20.68†	21.16*	21.45	21.05	22.73
	Top	16.34†	17.62*	17.93	17.39†	17.51*	17.87	18.05	18.63
	Root	4.09	3.88	3.87	3.29*	3.64	3.58	3.90	4.10
1/18 K	Total plant	15.26	14.63	14.92	14.70	15.08	14.94	14.80	15.06
	Top	12.03	12.05	12.32	11.99	12.41	12.22	12.23	12.28
	Root	3.23	2.58	2.60	2.72	2.67	2.71	2.57	2.78

* Significant difference at 5 per cent probability from control at same nutrition level.
† Significant difference at 1 per cent probability from control at same nutrition level.

When the response of the 9/9 K and 18/9 K levels to indoleacetic acid is compared by dry weight of total plant, differences appear. The 18/9 K level yields a significant increase over its control at 10^{-9} concentration and a decrease at 10^{-5}. The 9/9 K trend has only the significant decrease at 10^{-5}. Obviously more potassium was available in the 18/9 K level than in the 9/9 K level during growth of the plants. Although neither showed decided differences in growth of controls, this higher amount of potassium at the 18/9 K level may have been in part responsible for the increase of growth resulting from applications of indoleacetic acid. The two nutrient levels which in experiment II showed little difference in growth of controls and yet different response to indoleacetic acid were the 9/9 K and the 3/9 K. Of these the 9/9 K level yielded the significant increases. Because of much less growth in this test, a similar relationship between potassium supply and plant

growth may have existed in this 9/9 K level as in the 18/9 K level just described. In both cases the dry weight increases correlated with indoleacetic-acid treatment were associated with nutrition levels of relatively high potassium.

The facts that all indoleacetic-acid treatments at the 3/9 K level fall below their control, and that scattered points in this curve are significantly below, suggest that this growth substance may hasten the appearance of potassium deficiency. Symptoms of this deficiency were just beginning to be apparent at harvest, but observations were not recorded on their relative intensity in treated and control plants. No decided differences were obtained between the indoleacetic-acid treatments and control at the 1/18 K level.

TABLE 3

DRY WEIGHT OF TOTAL PLANT, TOP, AND ROOT. FIGURES REPRESENT MEAN WEIGHT IN GRAMS OF SIX SAMPLES OF EIGHT PLANTS EACH

NUTRITION LEVEL AND PLANT PART		NAPHTHALENE-ACETAMIDE CONCENTRATION							CONTROL (NO ACETAMIDE)
		10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}	10^{-10}	10^{-11}	
9/9 K	Total plant	7.41†	20.22†	23.06	23.44	24.24	23.83	.....	23.62
	Top	3.52†	13.63†	17.92†	18.70	19.36	19.45	.....	19.13
	Root	3.89*	6.61†	5.14*	4.74	4.88	4.38	.....	4.49
3/9 K	Total plant	7.27†	16.40†	21.93	22.60	21.87	21.83	21.79	21.87
	Top	3.47†	10.56†	17.00	17.90	17.60	17.55	17.57	17.52
	Root	3.80	5.84†	4.93	4.72	4.26	4.28	4.22	4.35
1/18 K	Total plant	6.34†	13.54†	14.75	16.27	15.93	15.30	16.19	15.46
	Top	3.00†	8.82†	11.50*	12.78	12.52	12.27	12.73	12.34
	Root	3.34	4.72†	3.25	3.49	3.40	3.03	3.47	3.12
Minus K	Total plant	5.80†	11.09*	11.63	12.10	12.19	11.75	12.30	12.30
	Top	3.05†	6.88†	8.84	9.62	9.60	9.39	9.95	9.64
	Root	2.76	4.22†	2.79	2.48	2.59	2.37	2.35	2.66

* Significant difference at 5 per cent probability from control at same nutrition level.
† Significant difference at 1 per cent probability from control at same nutrition level.

ALPHA NAPHTHALENE ACETAMIDE

EXPERIMENT IV.—This experiment was carried out in the greenhouse under similar conditions and at the same time as experiment III. Experiment IV had thirty-one treatments randomized in six blocks, replicated six times. Potassium levels of 9/9 K, 3/9 K, 1/18 K, and minus K were employed, as were acetamide concentrations of 10^{-5} to 10^{-11} (table 3). All plants at the 10^{-5} concentration were much stunted and after eight applications of the nutrients and growth substance had practically ceased growth. The experiment was therefore harvested on the twenty-ninth day after planting.

A pronounced downward curling of the primary leaves at all 10^{-5} concentrations occurred shortly after the first application of naphthalene acetamide. This per-

sisted permanently throughout the experiment, and these plants, regardless of potassium level, remained stunted. Their second internodes elongated little and only one small trifoliate leaf, if any, expanded. Plants at all other concentrations at a given potassium level had the same number of leaves as the control at that level, although there were noticeable reductions in leaf size and stem and petiole elongation at the higher concentrations.

Potassium deficiency in this experiment, as in the others, was first indicated by the pendant position of the primary leaf blades during the day. By the time of harvest chlorosis due to potassium deficiency was evident in the second trifoliate leaves in the minus K plants. Although the flower buds were still extremely small at harvest, there appeared no hastening of their development at this stage by any of the naphthalene-acetamide treatments.

Average dry weights of total plant, top, and root were analyzed (table 3). Differences between means of dry weights greater than 1.14 gm. for total plant, 0.83 gm. for tops, and 0.58 gm. for roots are significant at 5 per cent probability. At 1 per cent probability these mean differences must be greater than 1.50 gm. for total plant, 1.10 gm. for tops, and 0.76 gm. for roots.

The control treatments provide a basis for judging the effect of the four nutrient levels without any naphthalene acetamide. The total plant average weights and averages for tops both differ sufficiently so that each level is clearly separated from the others. The dry weights of roots fall into two groups. The 9/9 K and 3/9 K levels cannot be separated between themselves but they do differ significantly from the 1/18 K and minus K levels, which in turn cannot be separated. Yet the weights for roots decrease consistently with decreasing potassium, so the four levels appear to constitute four distinct nutritional conditions.

Examination of the significant differences between the naphthalene-acetamide treatments and the control at any given level shows that these differences are present only at the higher concentrations. For total plant dry weight they are only decreases, and the same is true for dry weight of tops. The only significant increases in the entire experiment are for dry weight of roots, and these increases are well localized at the 10^{-6} concentrations. At harvest the roots at these treatments were more fibrous and extensive than at others.

The response at any one nutrition level is the same in general trend as that at any other of the four levels tested (fig. 6). The difference appears only in degree, in that the weights are progressively less as potassium supply decreases.

The percentage increase in dry weight of root at the 10^{-6} concentration of naphthalene acetamide over the control at the same level is 47.2 at 9/9 K, 34.3 at 3/9 K, 51.3 at 1/18 K, and 58.6 at minus K. These increases occur in treatments associated with reduction in dry weight of tops. This decidedly alters the ratio of

tops to roots. At the 9/9 K level the dry weight of tops of the control treatment is approximately 4.3 times that of the roots. At the same nutrition level, but with naphthalene acetamide supplied in the nutrient at 10^{-6} concentration, the dry weight of the tops is only 2.1 times that of the roots.

Discussion

When different responses are obtained in nutritional work in which the solutions have been altered by the substitution of a salt, the question arises as to whether the effects are attributable to the altered essential element or to the substituted ion. Such inquiry may well be made in the experiments with indoleacetic acid reported here. The only nutrient level in experiment II which gave significant increases with indoleacetic-acid treatment was that of 9/9 K, which received no sodium after treatments commenced. Perhaps sodium prevented a similar response at the other nutrient levels where it was used as a substitute ion for potassium. But in experiment III, with much greater plant growth, the 9/9 K level failed to give significant increases. Presence of sodium could not have resulted in this response. In this experiment the 18/9 K level, with chlorine as a foreign ion and with higher potassium, did yield a significant increase in dry weight. Yet the increases of experiment II at 9/9 K were obtained on plants watered with solutions containing no chlorine. Sodium and chlorine may influence the response, but it seems unnecessary to consider the absence of sodium or the presence of chlorine of particular importance in obtaining increased growth with indoleacetic-acid applications.

The evidence seems to favor the view that variation in potassium supply rather than variation in the substituted or added ion was chiefly responsible for the different reactions at the several levels. If this is granted, it appears that relatively high potassium is necessary to obtain stimulating effects on growth of the total plant with indoleacetic acid. High potassium plants may be limited in growth by a relative deficit of growth substance. The addition of indoleacetic acid may raise this limitation and increased growth result. At the lower potassium levels this chemical element may be the limiting factor; thus greater supply of indoleacetic acid results in no growth increase.

GRACE (**5**) has studied the effect of several chemicals used with indoleacetic acid in a talc carrier on the rooting of stem cuttings. He concluded that potassium acid phosphate in combination with indoleacetic acid has decided effects on rooting.

In these experiments, whenever stimulation of total plant dry weight resulted from indoleacetic-acid treatment, it was observed in the same general region of concentrations, 10^{-7}, 10^{-8}, and 10^{-9}. Yet the much different response over the

entire range of concentrations suggests the value of employing several concentrations in experiments with growth substances.

Studies of the response of kidney bean plants to emulsion sprays of naphthalene acetamide have been made by KRAUS and MITCHELL (**7**) and by MITCHELL and STEWART (**10**). The latter observed the effects of four concentrations of emulsion spray as judged by dry-weight analysis 10 days after spraying. They reported root increases of 48 per cent with the most concentrated spray. Both papers mentioned the more fibrous nature of the roots and the suppression of top growth with concentrated sprays. These observations are similar to those reported in this paper for the 10^{-6} concentration, although in this work the naphthalene acetamide was applied in the nutrient solution and the plants were not harvested until flower buds were evident. No data were obtained concerning the relative efficiency of the increased root system of plants treated with naphthalene acetamide.

Summary

1. Red kidney bean plants grown in sand culture at different levels of potassium nutrition and receiving indoleacetic acid or alpha naphthalene acetamide in the nutrient solution in a series of concentrations were compared by dry weights of top, of root, and of total plant.

2. Plants receiving different nutrition but externally appearing alike responded in a markedly different manner to indoleacetic-acid treatment.

3. Relatively high potassium in the nutrient solution was necessary to obtain increased dry weight of total plant by indoleacetic-acid treatment.

4. Under certain levels of nutrition, increases as great as 12.5 per cent in total plant dry weight were obtained by indoleacetic acid supplied at the 10^{-9} concentration. Significant increases were obtained at concentrations of 10^{-7}, 10^{-8}, and 10^{-9}.

5. Indoleacetic acid appeared unable either to replace potassium or to enhance its utilization under deficiency conditions, as judged by total plant response.

6. The time of flowering was not hastened by indoleacetic-acid treatment, nor was there any indication that naphthalene acetamide accelerated the appearance of flower buds.

7. The number of leaves per plant was not increased by either growth substance, although leaf size varied considerably.

8. Percentage dry weight increased as potassium decreased, and only at relatively high concentrations of indoleacetic acid (10^{-4}) was increased succulence noted.

9. Plants at four potassium levels yielded the same general trend of response to naphthalene-acetamide treatment. They differed only in the degree of this response, in that dry weights were less with decreasing potassium.

10. Dry weights of roots increased 34–58 per cent at the 10^{-6} concentration of naphthalene acetamide. At this same concentration growth of tops was significantly reduced.

11. The first visible potassium deficiency symptom was expressed by the primary leaf blades retaining the nocturnal pendant position during the day.

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ANATOMY AND SORUS DEVELOPMENT OF CYSTOPTERIS BULBIFERA

BARBARA F. PALSER AND VIRGINIA F. BARRICK

(WITH TWENTY-FIVE FIGURES)

Introduction

The genus *Cystopteris* has given difficulty in classification. DIELS (**5**) placed it in the Woodsieae, but SCHLUMBERGER (**8**) excluded it from this tribe. BOWER (**1**) placed the genus among the Genera Incertae Sedis, but states that *Cystopteris* ranks nearer the Cyatheoid than the Davallioid derivatives, and that its origin is superficial as in the Woodsioid and Dryopteroid ferns. In a later publication (**2**) he states that the question of ancestry is between the Pteroid type with marginal, or the Cyatheoid type with superficial sori. More recently CHRISTENSEN (**4**) has placed *Cystopteris* in the Asplenioideae, between the Blechnoideae and Dryopteroideae.

Previously only two species, *Cystopteris fragilis* (L.) Bernh. and *C. montana* (Lam.) Bernh., have been examined morphologically and anatomically. The present investigation of *C. bulbifera* (L.) Bernh. was undertaken in the hope that further knowledge of the anatomy and morphology of this species would aid in establishing the relationships of the genus.

Material and methods

Leaves and rhizomes were collected at the base of the eastern side of Mount Toby, Massachusetts. Material collected in May was used for studying the origin and development of the sorus. The young fronds were only partly unfolded; the tips which bore very young sori were still encased by the rest of the frond. Material collected in October was used for studying the rhizome and root. Portions of root, rhizome, petiole, pinnae, and young leaves were killed and fixed in a chromo-acetic solution of 1 per cent chromic acid and 2 per cent acetic acid and were then imbedded in paraffin. Most sections were stained in safranin-light green or in safranin-gentian violet combinations. Sections of early stages of the sorus were stained in Heidenhain's iron-alum haematoxylin and counterstained in safranin.

Observations

RHIZOME.—The rhizome develops radially, with leaves diverging from all sides. Because of the closeness of the leaf bases to one another, the rhizome gives the appearance of being merely a spiral succession of imbricated bases. Although the

greater part of the rhizome is made up of leaf bases, the central portion contains a dictyostele with curving meristeles bordering the long leaf gaps. Transverse sections of the rhizome show the number of meristeles ordinarily to be either three or four, although there are occasionally five. The binary leaf traces found in the leaf base arise individually, each from a separate meristele. Each leaf is accompanied by a single root. The trace which enters the root diverges near the base of the leaf gap. This base is formed by the divergence of a single meristele into two strands, each of which may give off a leaf trace before anastomosing with an adjacent meristele (fig. 1, rt_1, lt_1), or—more often—one will give off a leaf trace before anastomosing but the other will merge with an adjacent strand before giving off a leaf trace (fig. 1, rt_4, lt_4). The leaf traces, usually diverging in the lower half of the leaf gap, do not always arise at the same distance from the base of the gap, nor at the same level.

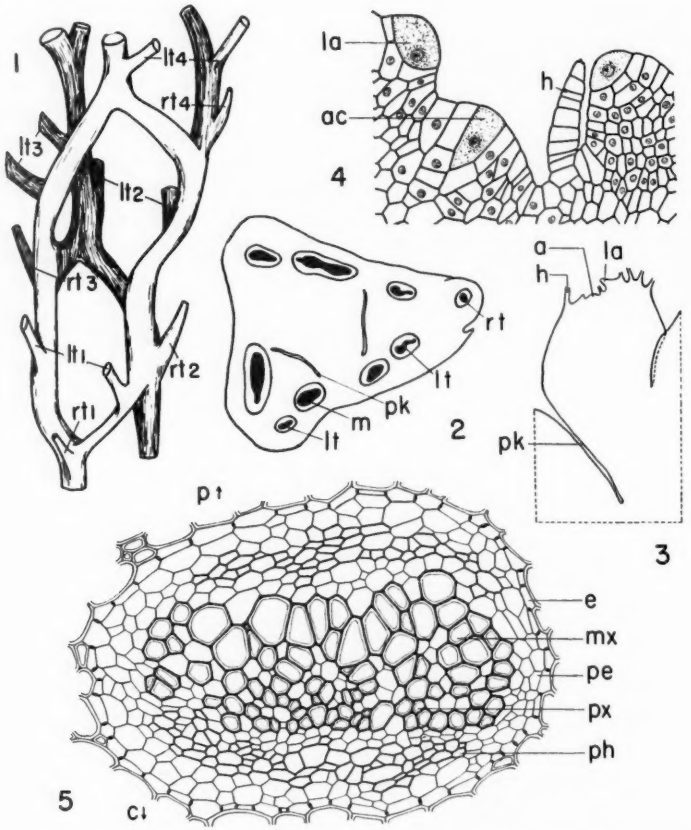
Pockets in the rhizome are formed between the adaxial side of the leaf base and the rhizome itself, and extend down into the pith region, often extending below the point at which the leaf traces diverge from the meristeles into the leaf base (figs. 2, 3). GWYNNE-VAUGHAN (**7**) describes similar pockets in *C. fragilis* as large, downward ranging into internal ground tissue, and limited to three in any cross section.

The apex of the rhizome is protected by scales, multicellular hairs, and glandular hairs, as well as by the petioles of young leaves. The large apical cell is wedge-shaped, with three cutting faces (fig. 4). Each meristele is surrounded by a continuous endodermis, the cells of which show a Casparian strip on the radial walls. A pericycle, two or three cells thick, lies between the endodermis and the phloem. The central core of xylem, oval in transverse section, is surrounded by parenchyma and, outside of that, by a band of phloem which is not always continuous. The isolated protoxylem cells are found near the cortical side of the xylem core, the larger metaxylem cells being toward the pith.

Each vascular strand of the leaf trace resembles a meristele but differs in the shape of the xylem strand. In transverse section the two traces are mirror images, with the protoxylem regions at the two ends of the traces partly inclosed by hooks of metaxylem (figs. 6, 7). In the rachis the two leaf traces merge, making a V-shaped trace, although the xylem strands retain in part their characteristic shape (fig. 8).

ROOT.—The root trace diverges endogenously from a meristele, usually below but sometimes above the leaf gap. The primordium of a root may be seen near the rhizome tip, close to the base of the primordium of the leaf. The first protoxylem cells of the diarch stele appear after the root has progressed through the cortex far enough to break the epidermis. The course that the root may take when it is passing through the cortex of the rhizome varies. The root may grow obliquely

toward the tip but before becoming free turn back; or it may pass outward almost at right angles. When it breaks through the epidermis it may grow parallel to and in contact with the rhizome for some distance. The root cap is seven or eight cells thick just beyond the apex.

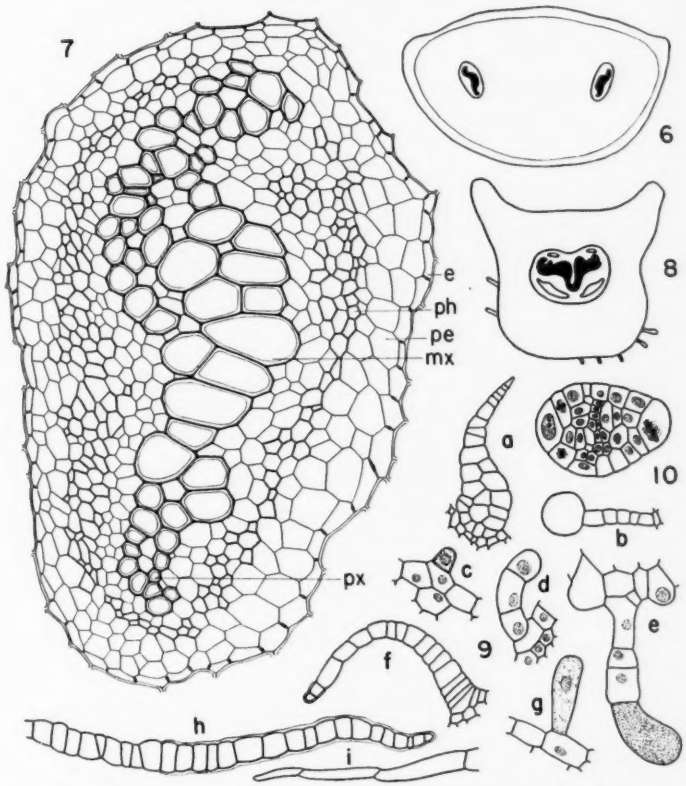


FIGS. 1–5.—Fig. 1, reconstructed vascular system: *lt*, leaf trace; *rt*, root trace. Fig. 2, diagram of cross section of mature rhizome. Fig. 3, same of longitudinal section of rhizome through apex: *a*, apex; *la*, leaf apex; *h*, hair or scale; *pk*, pocket; *m*, meristele. Fig. 4, apex of rhizome: *ac*, apical cell. Fig. 5, detail of meristele: *mx*, metaxylem; *px*, protoxylem; *pe*, pericycle; *e*, endodermis; *p*, region of pith; *ph*, phloem; *c*, region of cortex.

DERMAL APPENDAGES.—Types of appendages found on the sporophyte are scales, multicellular hairs, glandular hairs, and unicellular hairs (fig. 9). They occur on young parts as on the rhizome tip, petioles, rachis, and pinnae of young leaves, and on both the inner and outer surface of bulbils. They generally dis-

appear as these parts mature, but unicellular hairs—which may be slightly swollen at the tip—persist on the lower surfaces of the midribs of pinnae and pinnules.

LEAF.—The leaves are long, delicate, and light green in color. The mesophyll does not have a differentiated palisade layer, but there is more intercellular space

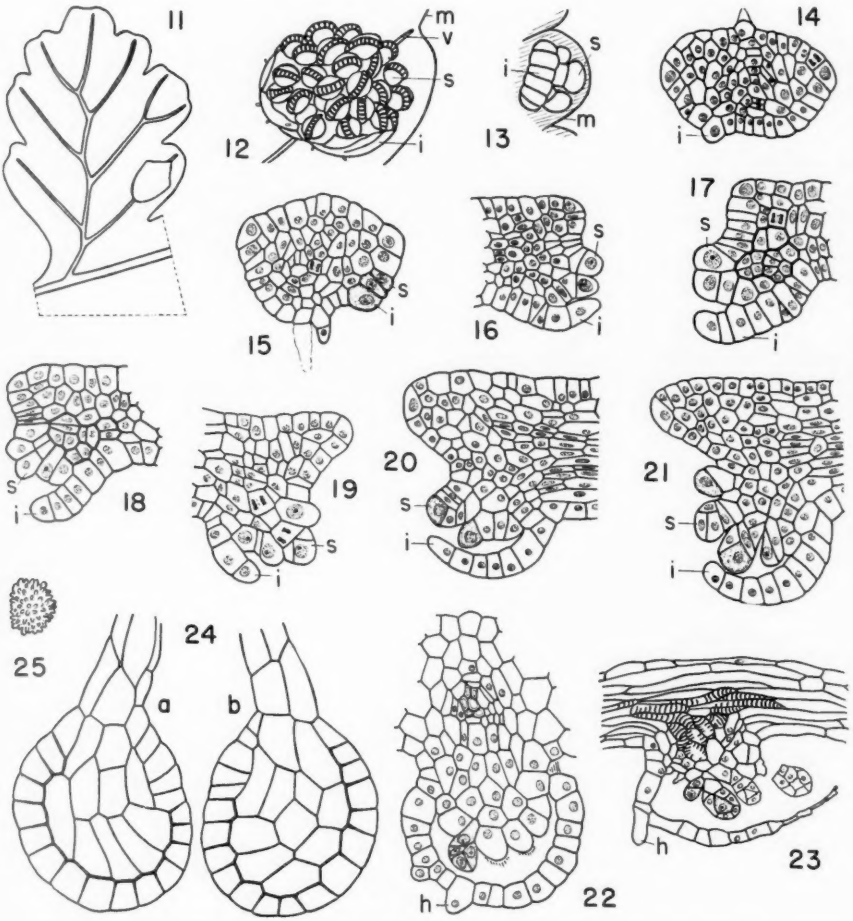


FIGS. 6–10.—Fig. 6, diagram of cross section of stipe. Fig. 7, detail of vascular bundle of stipe: *e*, endodermis; *ph*, phloem; *pe*, pericycle; *mx*, metaxylem; *px*, protoxylem. Fig. 8, diagram of cross section of rachis. Fig. 9, dermal appendages (*a–i*). Fig. 10, cross section of very young pinnule.

in the lower part than in the upper. Leaf primordia are very plump, as are also the pinnae as they arise from the rachis. The margin of each pinnule is composed of a row of cells with two cutting faces, which by continued divisions bring about the broadening of the pinnule (fig. 10). The pinnules bear two to eight sori near the outer margin of the small lobes. The sori appear while the pinnule is subterete.

SORUS.—In a mature leaf the sorus is seated near the margin of a pinnule on a

vein which extends beyond it. When young, the sorus is covered by a pocket-like indusium which opens on the side toward the margin of the pinnule (fig. 11). As soon as the first sporangia mature, the indusium begins to shrivel and eventually



FIGS. 11–25.—Fig. 11, pinnule with young sorus. Fig. 12, mature sorus: *m*, margin of leaf; *v*, vein; *s*, sporangium; *i*, indusium. Fig. 13, very young sorus, surface view. Figs. 14–21, stages in the development of sorus. Fig. 22, section cut across vein through young sorus: *h*, hair. Fig. 23, section cut parallel with vein through young sorus. Fig. 24*a*, *b*, two faces of young sporangium. Fig. 25, mature spore.

exposes all the sporangia (fig. 12). As it shrivels it is apt to tear lengthwise, giving the appearance at maturity of a basal indusium made up of scales. The indusium bears unicellular hairs and is one cell in thickness, except at the base directly in the

center where it may be two cells thick. The bilateral symmetry of the sorus is exaggerated by lateral pouches of the indusium, which extend outward from the base and over the top of the receptacle (fig. 22). The axis of the receptacle is inclined toward the margin of the pinnule (fig. 23).

The first indication of the sorus is found while the pinna is composed of undifferentiated tissue and has broadened but slightly (figs. 14, 15). As seen in section, an initial of the indusium five or six cells back from the margin becomes enlarged and papillate. This is followed by two or three sporangial initials between the indusium and the marginal cell. Two or three anticlinal divisions may occur in the superficial cells between the sporangial initials and the margin, but these cells do not enlarge much at this time (figs. 16–18). The tissue of the receptacle is formed by repeated division of the cells just beneath the sporangial and indusial initials (figs. 17–19). The cells of the young indusium undergo periclinal divisions, forming a layer curving from the proximal side of the sorus toward the margin (figs. 13, 16–21). In early stages of the sorus, marginal growth of the leaf is practically at a standstill and the marginal cell is often forced by intercalary divisions in the soral region to a position almost on the plane of the upper surface of the leaf (figs. 16–18). Growth of the receptacle is greater on the proximal side of the sorus, and the new sporangial initials are formed between the oldest sporangium and the base of the indusium (figs. 16, 18, 20). One, rarely a second, sporangium is formed on the distal side of the receptacle (fig. 21). This shows a gradate sequence in sporangial development, modified by the suppression of receptacle growth on the distal side of the sorus. The sorus later becomes mixed. The marginal cell at all times remains completely distinct from the tissues of the sorus (figs. 16–18), although in early stages the growth of the receptacle on the proximal side, the more advanced development of sporangia on the distal side, and the suppression of activity in the marginal cell—all place the young sorus on what has been interpreted to be the margin of the leaf. By the time there are several sporangia in early stages of development, the more active marginal growth of the leaf places the sorus on the under side of the leaf (figs. 19–21). About this time maturation of the main vein of the leaflet begins, followed by differentiation of the lateral veins. In the early stages, provascular strands can be seen extending into the receptacle of the young sorus and into the leaf blade beyond (fig. 20).

Either the first or second division of the sporangial initial is usually oblique. The mature sporangium has a slightly oblique annulus of fourteen indurated cells interrupted by the three-rowed stalk. The cell between the base of the annulus and the stalk is longer than the annulus cells, and the walls are unthickened. The mature stalk is relatively stout, with irregularly inclined walls (fig. 24). The sporangium contains twenty-four to forty-eight spores, but the usual number is thirty-two. They are dark brown, without a perispore, and are covered with spine-like protruberances (fig. 25).

Discussion

In considering the relationships of *Cystopteris* the following points should be considered: its various vegetative characters—delicate habit, crowded leaves, dermal appendages, dictyostelic stem structure, and binary leaf traces with which one root is associated; its reproductive characters—the superficial position and origin of the sorus, its zygomorphic form, the pocket-like indusium, the gradate sequence changing to mixed, the three-rowed stalk of the sporangium, and the bilateral spore.

There is little that suggests relationship between *Cystopteris* and any group of the Marginales. The only point which would ally them is a marginal origin of the sorus. In *Cystopteris* the sorus is seated on a vein which sends a branch into the receptacle but continues beyond toward the leaf margin. In forms secondarily superficial (for example, *Davallia*) the origin of the sorus is definitely marginal on a flattened margin receptacle; the superficial position is assumed when the sporangia are somewhat well developed. In almost all cases the marginal cell of the leaf is active in the formation of the receptacle, sporangia forming on both sides of it; and the continuation of the lamina as upper indusium is a proliferation of the leaf tissue on the upper side of the marginal cell (1). In *Cystopteris*, however, the marginal cell continues to function as the marginal cell of the leaf and is not concerned in the formation of the sorus.

BOWER (3) finds that "the segmentation of the sporangia [in *C. fragilis*] is also a matter for note. It will be seen that the stalk is a relatively massive one with irregularly inclined walls. This . . . . is in sharp contrast to the simple transverse segmentation of the stalk seen in *Davallia*."

The interpretation of the origin of the sorus as marginal may be attributed to the precocity of the pinnule in its soral development, since in ferns where the sori are obviously superficial (for example, *Asplenium*) the pinnule may become broadened and have a well-defined vein before the sporangia appear (1). In *Cystopteris* the development of the first sporangium precedes the broadening of the leaf and the development of the vascular tissue. At this stage the room for the sorus on the under surface is very limited. As the leaf broadens the almost marginal position is maintained for a short period by the great activity in the sorus in comparison with the slight activity of the margin, but the marginal cell at all times remains distinct from the sorus. The superficial position is definite before the vascular tissues are mature. The origin and development of the sorus have been observed by GOEBEL (6) in *C. montana* and by BOWER (3) in *C. fragilis*. Their figures indicate that a similar interpretation is reasonable for these two species.

In the Superficiales similarities can be found to members of the Onocleoideae, the Dryopteroideae, the Asplenioideae, and the Woodsieae. The smaller forms of the Dryopteroid group have a simplified dictyostele, and certain species (for ex-

ample, *D. phegopteris* and *D. thelypteris*) have a binary leaf trace (1). The receptacle of the sorus in this group is horseshoe-shaped, and while there may be a very early tendency to gradate sequence of the sporangia, it is quickly replaced by a mixed condition. The sporangium has not lost all traces of the obliquity of the annulus, but it is not so noticeable as in *Cystopteris*. The stalk is three-rowed. *Dryopteris* differs from *Cystopteris* in the accentuated tilting and zygomorphy of the receptacle and in the early mixed sporangial sequence.

CHRISTENSEN's most recent classification (4) places *Cystopteris* in the Asplenioideae, and suggests a relationship to *Athyrium*. The adult stem of *Athyrium* contains a rather wide-meshed dictyostele. The petiole is traversed at its base by broad straps of the leaf trace, which unite below the blade to form a gutter-shaped meristele (1). The leaves are borne spirally. *Cystopteris* shows close resemblance in anatomy but differs in soral structure and in details of the sporangium, which has an annulus with an oblique tendency and relatively massive stalk in contrast to the incomplete vertical annulus and one-rowed stalk of the sporangium of *Athyrium*.

In the appearance, size, and texture of the fronds and in anatomy, *Cystopteris* shows a close resemblance to the Woodsieae. The vascular system of *Woodsia* is a simple dictyostele, with two leaf trace strands, with which one or more roots are associated (8).

Cystopteris forms a link in the series tracing the zygomorphy of the sorus from the complete radial sorus with basal indusium—found in some species of *Woodsia*—to the horseshoe-shaped sorus with the tilted receptacle and covered by an incomplete indusium found in *Dryopteris*. In the Woodsieae and *Dryopteris* the slightly oblique annulus is interrupted by the three-rowed stalk, and the spore output is 48–64. In the Woodsieae the sequence of sporangial development is initially basipetal, later becoming mixed as in *Cystopteris;* in *Dryopteris* the gradate sequence is merely suggested. *Cystopteris* seems to be intermediate between *Woodsia* and *Dryopteris*, but more closely allied to *Woodsia* than to *Dryopteris*.

Summary

1. The rhizome of *Cystopteris bulbifera* is radial, with leaves diverging from all sides. It contains an unperforated dictyostele with curving meristeles bordering the leaf gaps. In transverse section ordinarily three or four meristeles are found.

2. The strands of the binary leaf trace diverge individually in the lower half of the leaf gap, one from each side of the leaf gap, and merge in the rachis.

3. Each root arises singly and accompanies the leaf base, the root trace diverging near the base of the leaf gap.

4. Unicellular, multicellular, and glandular hairs, and also scales are present on

young parts of the sporophyte. Unicellular hairs persist on the lower surface of the rachis and of the midribs of the pinnae and pinnules.

5. The sorus originates on the pinnule while it is subterete. The sorus is superficial in origin; the indusium and sporangial initials appear a few cells back from the marginal cell, which continues its marginal activity and takes no part in the formation of the sorus. The sorus is seated anterior to the vein ending. Its receptacle has bilateral symmetry and is tilted toward the margin of the pinnule.

6. The sporangium has a slightly oblique annulus, which usually consists of fourteen cells interrupted by a three-rowed stalk. The sequence in development in the sorus is at first gradate but later mixed.

7. The closest affinities of *Cystopteris* seem to be with the Woodsieae.

The writers express their thanks to Professor ALMA G. STOKEY for helpful direction and criticism during the course of this study.

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GRASS STUDIES. V. OBSERVATIONS ON PROLIFERATION[1]

ETLAR L. NIELSEN

(WITH SIX FIGURES)

Introduction

ARBER (1) defines proliferation in grasses as "the conversion of the spikelet, above the first glumes, into a leafy shoot." Reports covering a number of species wherein proliferations have been observed have been published (1, 2, 3, 5). The majority of these observations appear to have been made upon plants growing in areas of high altitudes, high latitudes, or in moist situations. The present study was made mainly upon plants growing under the relatively xeric conditions in northwest Arkansas during the summer months of the last few seasons, under very wet conditions of west-central Wisconsin, under greenhouse conditions, or under grazing.

Observations

FESTUCA OBTUSA SPRENG.—A plant upon which a number of florets were proliferating was collected near Crosses, Madison County, Arkansas, in July, 1936. Subsequently other material of this same species bearing abnormal spikelets was collected from central and northwest Arkansas. With one exception the plants grew in dry and open situations. Examination of the cited herbarium material indicates that some spikelets of the panicles appear normal in every respect while others bear proliferating florets. In some spikelets only one floret developed. This proliferated and was subtended by a pair of glumes. In other instances, where two or more florets had developed, the lowermost appeared normal while the more distal member of the same spikelet had proliferated. No spikelets have been observed with more than one floret proliferating, however, nor were any normal florets observed to be borne more distal than the proliferating one (fig. 1).

Herbarium material.—Crosses, Madison Co.: *Nielsen*, no. 4045. Mt. Magazine, Logan Co.: *Nielsen* and *Younge*, no. 5952. White Rock Mountain, Franklin Co.: *Nielsen*, no. 6016. Devils Den State Park, near Winslow, Washington Co.: *Younge* and *Nielsen*, no. 5959 (Mr. J. R. Swallen comments concerning this specimen: "The one or two apparently normal spikelets seem to be *Festuca versuta* Beal. . . . . However the plant is abnormal and since *F. obtusa* Spreng. is common in this region I believe it is more likely to be the latter species.").

BROMUS INERMIS LEYSS.—In this species essentially the same conditions ob-

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FIGS. 1–6.—Fig. 1, *Festuca obtusa*, proliferating and normal spikelets. Fig. 2, *Bromus purgans*, much enlarged spikelets but proliferations not well developed morphologically. Fig. 3, *B. inermis*, proliferations wholly replacing normal spikelets. Fig. 4, *Phleum pratense*, well-developed proliferations of spikelets replacing normal structures. Fig. 5, *Avena sativa*, spikelets with proliferating upper florets. Fig. 6, *Panicum virgatum*, proliferation that replaced entire panicle.

tained as were found in *Festuca obtusa*. In 1938, proliferation was observed in nine of twelve selections of smooth brome grass growing in the grass nursery at the Agricultural Experiment Station, Fayetteville, Arkansas. Nearly all the spikelets of the panicle of some plants were abnormal in development, whereas in other panicles of plants of the same selection only a few florets appeared to be affected. In most instances only a single floret of a spikelet proliferates. Although these selections have been under observation since 1937, this condition was observed in this species in 1938 only. Shortly after July 10, 1938, when this condition was observed at Fayetteville, the writer examined a number of areas of this species in Minnesota and Wisconsin. No proliferations were found at this time, nor were any observed in the aftermath growth on these same areas when subsequently examined on September 17 of the same year (fig. 3).

Herbarium material.—Grass nursery, University of Arkansas Experimental Farm, Fayetteville: *Nielsen*, no. 5595.

BROMUS PURGANS L.—During the fall of 1939 much-enlarged florets occurred abundantly on plants of this species situated on dry ledges and in exposed positions in a number of localities of northwest Arkansas. In none of the numerous plants examined were the proliferations as well developed as in the selections of *B. inermis* previously discussed, and—in contrast to the condition in the latter species—usually all the florets of a spikelet were abnormal; in most instances comparatively few normal florets were found on the affected plants (fig. 2).

Herbarium material.—Lake Wedington, Washington Co.: *Younge* and *Nielsen*, nos. 6007, 6011. Devils Den State Park, Washington Co.: *Younge* and *Nielsen*, nos. DD200, DD204.

PHLEUM PRATENSE L.—Only in 1938 were proliferating spikelets observed in the nearly mature inflorescences in four of nineteen selections of this plant grown continuously in the grass nursery at the station during the period 1937–1940. The first abnormalities were observed on July 15, and others subsequently appeared until July 25. Prior to June 1, moisture had been plentiful. A period of light rainfall and high temperatures followed. Here the combination of abundant moisture followed by severe drought appeared associated with the occurrence of proliferation.

Also in 1938, aftermath timothy meadows in the vicinity of Chippewa Falls, Wisconsin, were examined regularly during September and October. No proliferations were found until October 21. Those observed at that time were found on plants growing near the margin of a meadow situated in a lowland subjected that year to frequent inundation, or along a service road entering the meadow. All the abnormal inflorescences were either emerging or had just emerged from the boot. During 1938 the rainfall at Chippewa Falls had been abundant during the entire summer and autumn, and moderate temperatures had prevailed. In this instance

the phenomenon may have been induced by the short days and low temperatures of late autumn (fig. 4).

Herbarium material.—Grass nursery, University of Arkansas Experimental Farm, Fayetteville: selections 361, 365, 393. Chippewa Falls, Chippewa Co., Wisconsin (October, 1938): *Nielsen* (five specimens).

AVENA SATIVA L.—A proliferating specimen of the commercial variety Ferguson 922 was forwarded to the writer by Mr. Paul H. Millar of the Arkansas State Plant Board. He reported the abnormality as "occurring abundantly in very moist or wet situations in the oat fields of the rice belt near Lonoke, Lonoke County." In a subsequent conversation Mr. Millar stated that he had not, at the time of collection, observed proliferations on plants growing on drier situations. In the modified spikelets examined either one or two florets were affected, the distal one of which was borne upon a much elongated rachilla which frequently reached 2 cm. in length and at times exceeded the somewhat enlarged subtending glumes (fig. 5).

Herbarium material.—Oat fields, Lonoke, Lonoke Co., Arkansas: *P. H. Millar* (May, 1939).

PANICUM VIRGATUM L.—During the three winters of 1937–1940 a number of plants of several selections of this species were grown in the greenhouse at this station. Some plants of selection no. 644 developed proliferations that entirely replaced inflorescences. These plants, grown from caryopses taken from plants growing along a railroad embankment a few miles north of Fayetteville, were planted in the greenhouse in early September, 1937. Growth of the seedlings appeared normal until after March 1, 1938, when the development of proliferation was first noted. By mid-April a few proliferations had reached the stage shown in figure 6. Some had produced several tillers, and adventitious roots had appeared on the short rhizomes. Plants of this no. 644 and of two other selections that have also proliferated under greenhouse conditions, no. 339 from O'Neil, Nebraska, and no. 1602 from Stillwater, Oklahoma, have since flowered normally and produced good yields of viable caryopses when grown under field conditions at the Experimental Farm, Fayetteville. On September 7, 1940, numerous proliferating plants of this species were observed in a pasture area. In this case the abnormalities developed only on those plants that had been retarded by the removal of their crowns through grazing.

A cytological examination of material taken from these selections of *Panicum* indicates no apparent reason to assume that in this species, at least, the vegetative mode of reproduction is necessarily associated with higher chromosome numbers (4, 5).

Herbarium material (taken from greenhouse-grown plants).—*Nielsen*, selection no. 644 (April, 1938, and March, 1939); no. 1602 (Jan. 9 and April 15, 1940); no. 339 (April 1, 1940). Northwest Arkansas Land Use Project, near Wedington, Arkansas: *Younge* and *Nielsen*, no. 6467.

Discussion

From the preceding observations and from many of those recorded in the cited bibliographies, it appears that adverse environmental factors, or abrupt changes of environmental factors—particularly moisture, light, and perhaps temperature—play an important part in the mode of reproduction of some of the numerous races of certain grass species. As indicated in the cited papers, form names have been applied in some instances to races showing tendencies toward proliferation. It seems advisable that such taxonomic description should be postponed until the physiological responses of a number of races within a given grass species have been studied under a wide range of controlled environmental conditions and the effects of such conditions upon mode of reproduction critically observed.

Summary

Proliferation has been observed to occur in *Festuca obtusa*, *Bromus inermis*, *B. purgans*, *Phleum pratense*, *Avena sativa*, and *Panicum virgatum*. The general external morphology of the proliferations is briefly described and the environmental conditions of the proliferating plants indicated.

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STRUCTURE OF STEMS IN RELATION TO DIFFERENTIATION AND ABORTION OF BLOSSOM BUDS[1]

B. ESTHER STRUCKMEYER

(WITH THIRTY-EIGHT FIGURES)

Introduction

It was shown by WILTON and ROBERTS (**6**) that differences exist in the anatomical structure of the stems of nonflowering and flowering plants. WILTON (**7**) later reported that the degree of cambial activity in annual plants depends upon whether they are vegetative, budding, or flowering. She observed that the cambium becomes less active as the plants form blossom buds, and by the time these buds have developed into flowers and fruits, cambial activity has ceased. The characteristics of the phloem tissue in stems of nonflowering and flowering plants were described by STRUCKMEYER and ROBERTS (**5**). They found the sieve tubes and companion cells to be fewer in number, thicker walled, and smaller in size in stems of flowering than in nonflowering plants. Callose was also more abundant in stems of a number of species of flowering plants.

In connection with these investigations, the following questions arose: (*a*) Do the anatomical changes in stems of plants placed in an environment favorable for flowering become apparent before the appearance of blossom primordia, or at the same time as blossom primordia, or are these changes unrelated to the differentiation of blossom primordia? (*b*) What is the effect of return to an environment favorable to vegetative growth upon plants previously subjected to an environment inducive to flowering?

Material and methods

The plants were grown in the greenhouses of the department of horticulture at the University of Wisconsin. The five species under observation were:

Compositae:	*Cosmos sulphureus* Cav. var. Klondike, *Xanthium echinatum* Murr.
Cruciferae:	*Mathiola incana* R. Br. var. Christmas Pink
Labiatae:	*Salvia splendens* Ker. var. Harbinger
Leguminosae:	*Glycine max* Merr. var. Biloxi

Several series, each composed of approximately seventy plants of these species, were placed in greenhouses with minimum temperatures of 55°, 65°, and 75° F., and are respectively designated as cool, medium, and warm. Long-day conditions

[1] Published with the approval of the Director of the Agricultural Experiment Station. This investigation was supported in part by a grant from the Wisconsin Alumni Research Foundation.

were secured by the use of electric lights employed from about sunset until midnight. These delivered to the plants 30–80 foot candles, as measured by a Weston photometer. The normal winter days of November, December, and January were used as short days. Before November and after January, the days were artificially shortened to $9\frac{1}{2}$–10 hours. Some of the plants placed on the short days were sampled at regular intervals while the remainder were returned to long days. The latter were also sampled after varying lengths of exposure.

Samples were taken of the fourth internode and of the stem tip of the same plants, three plants being taken at each sample date. The material was fixed in formalin-acetic-alcohol. Butyl alcohol was used as the dehydrating agent, and the material was imbedded in paraffin. Transverse and longitudinal sections 12–15 μ thick were cut. The sections were stained with 50 per cent alcoholic safranin and with light green in clove oil.

Observations

Plants requiring different environmental treatment for the formation of blossom buds were examined. Soybean, *Salvia*, *Cosmos*, and *Xanthium* initiate blossom buds when exposed to short days at appropriate temperatures (**1**, **2**, **3**, **4**). Stock produces blossoms under long-day conditions at a cool and medium temperature, and after a greater length of time on short days at a cool temperature.

Microscopic blossom primordia were evident on plants of soybean and *Salvia* when exposed to 9 short days and a warm temperature. Unless this short-day treatment was continued approximately 17 days, the primordia failed to develop into flowers and the plants gradually acquired vegetative characteristics. Blossom primordia were apparent on plants of *Cosmos* given 12 short days, and an exposure of at least 18 short days was necessary for these plants to retain their blossom buds and continue development if transferred to long days. The time necessary for development of blossom primordia is greatly dependent upon the temperature. Stock requires even more time for the formation of blossom primordia, for only after 18 days of long-day treatment at a cool temperature were microscopic primordia apparent. At the warm temperature, these plants remained vegetative on both long and short days. On the other hand, primordia were apparent on plants of *Xanthium* after 5 days of short-day treatment at a warm temperature.

These results indicate that under the most favorable environmental conditions microscopic blossom primordia are apparent only after varying lengths of treatment for different species of plants. Perhaps more important is the fact that unless this favorable environmental treatment is continued, even after blossom primordia are formed, they will not develop into blossoms but will abort. *Xanthium*, however, does not exhibit this condition. Two to three short days are sufficient

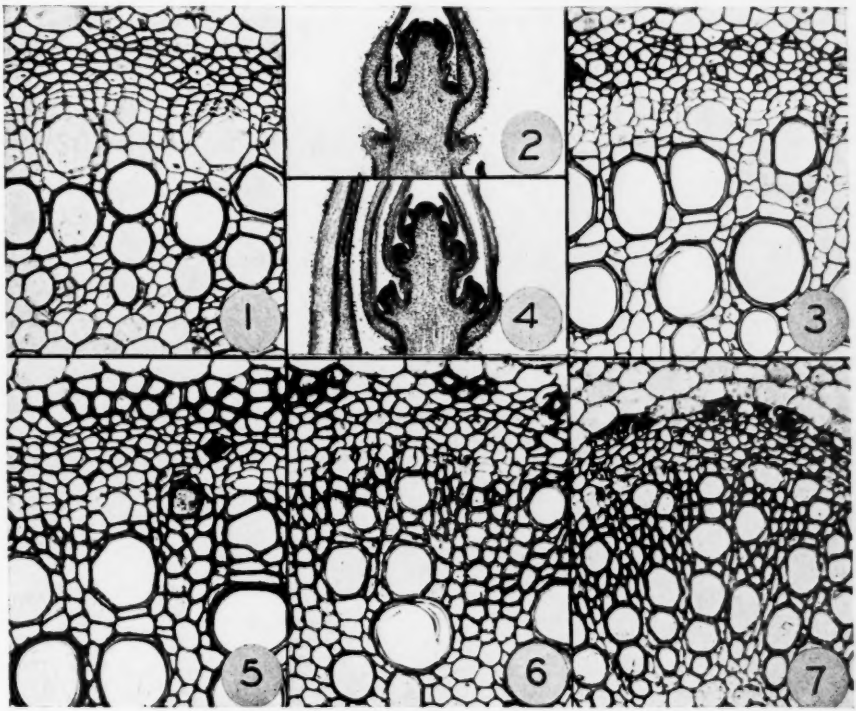
to induce this species to produce blossoms, even though the plants have been transferred to an environment favorable to vegetative growth. Staminate blossoms may be formed after plants are exposed to but one short day. Therefore soybean, *Salvia*, *Cosmos*, and stock require a certain period of exposure to a favorable environment for the initiation of blossom primordia, and this period must be continued for a length of time after primordia are formed if they are to develop into flowers.

The first question considered in this study is: When do the anatomical changes in the stem first become apparent in plants placed in an environment favorable to flowering? During this investigation it was observed that the transformations in anatomical structure were apparent soon after the beginning of a treatment inducive to flowering. *Salvia* illustrates this condition. Stems of plants given 5 short days displayed a less active cambium than plants that remained on long days at a warm temperature. The former also showed a decrease in the differentiation of number of xylem vessels, sieve tubes, and companion cells (figs. 1–3). Although alterations in the structure of the stem were apparent after 5 short days, microscopic blossom buds were not evident until 8–9 short days. Plants given 8 short days showed a further reduction in meristematic tissue. Little differentiation of tissue was in progress at this stage, although a number of the cells were becoming thicker walled (figs. 4, 5). In stems of plants given additional short days, the cambial region—which at this time was limited to one or two layers of cells—continued to give rise to xylem cells. Few if any phloem cells were being formed and these were composed chiefly of phloem parenchyma cells. Continued thickening of the walls of the vascular tissue and the pericycle was apparent (fig. 6). The stem tips showed progressive differentiation of the primordia into blossom buds. The stems of plants that had received 39 short days showed no active cambium. The sieve tubes and companion cells were smaller than those in the stems previously described (fig. 7). By this time the plant was in flower.

Similar changes in anatomical structure accompanying blossom initiation were observed for soybean, in that alterations in the structure of the stem were apparent after 6 short days while blossom primordia were not apparent until 9–12 short days at a warm temperature (figs. 8–12). Likewise for soybean, the differences in anatomical structure became more pronounced as the plants were given additional short days. Plants given 39 short days, and which by this time had macroscopic blossom buds, showed the absence of a cambium in the stem. There was no evidence of meristematic tissue and the cells had become increasingly thicker walled (figs. 14, 15).

The alteration of the anatomical structure in *Cosmos* closely resembles that already described for *Salvia* and soybean (figs. 18–22). *Xanthium* presents a similar condition. It responds more quickly to photoperiod at the proper temperature

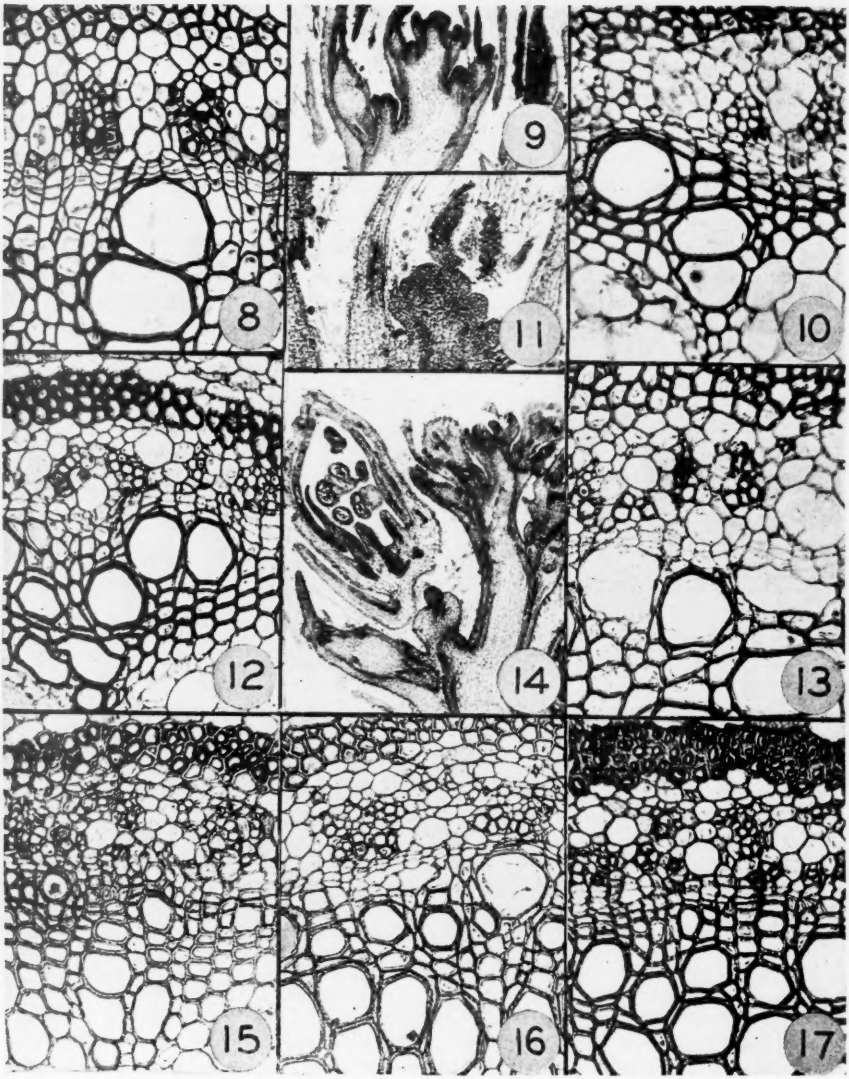
than do the other species studied. At a warm temperature, 2–3 short days are sufficient to induce this species to flower. Although blossom primordia are not present after 3 short days, they are evident on plants that have received 4–5 short days or 2 short days and 3–4 long days. Plants given 3 short days showed no distinguishable blossom primordia, but the structure of the stem had become altered



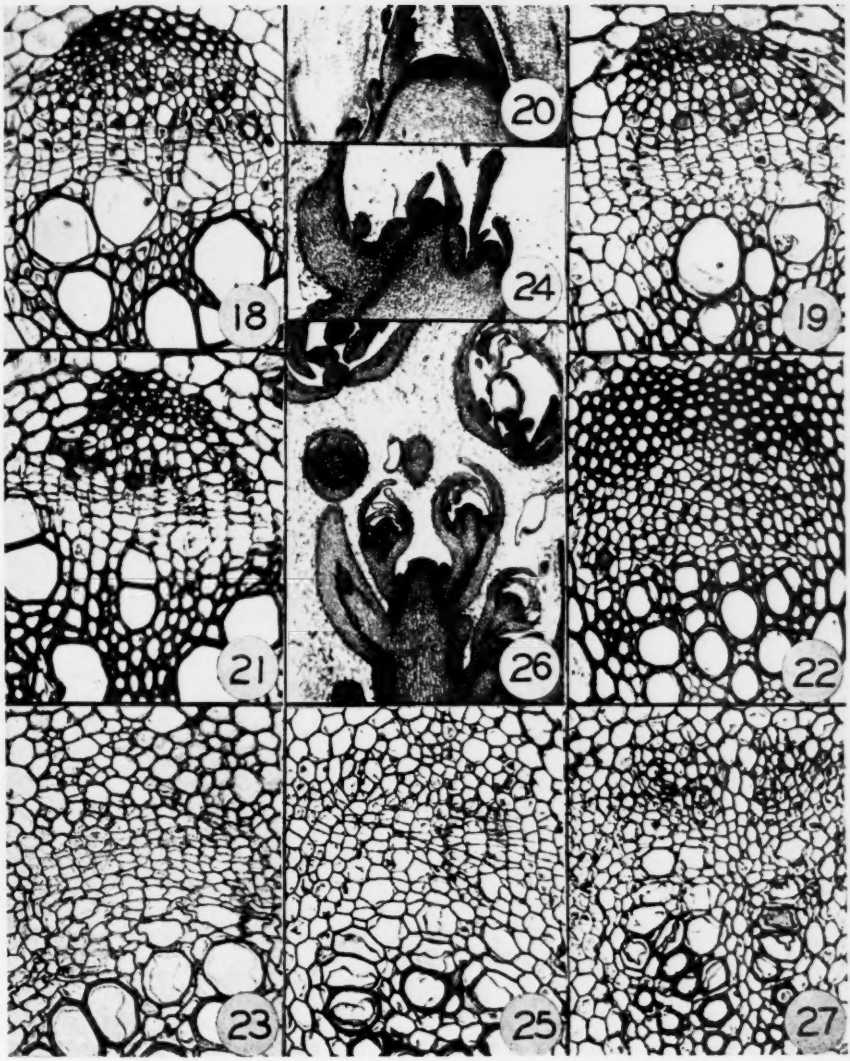
FIGS. 1–7.—Stem tips and transections of fourth internode of *Salvia:* Fig. 1, nonflowering plant; active cambium present. Figs. 2, 3, plant exposed to 5 short days; no apparent blossom primordia, less active cambial region, fewer xylem vessels differentiating, and phloem parenchyma rather than sieve tubes forming. Figs. 4, 5, 8 short days; prominent blossom primordia, cambium less active. Fig. 6, 22 short days; cells of stem thick walled, formation of vascular tissue almost ceased. Fig. 7, flowering plant; cells of stem small and thick walled, little or no meristematic tissue present.

(figs. 28–31). A less active cambial zone was apparent, fewer secondary elements were being formed, and the cells surrounding the xylem vessels were becoming thick walled. As the primordia developed into blossoms the cambium remained somewhat active, giving rise to new xylem vessels (figs. 32, 33).

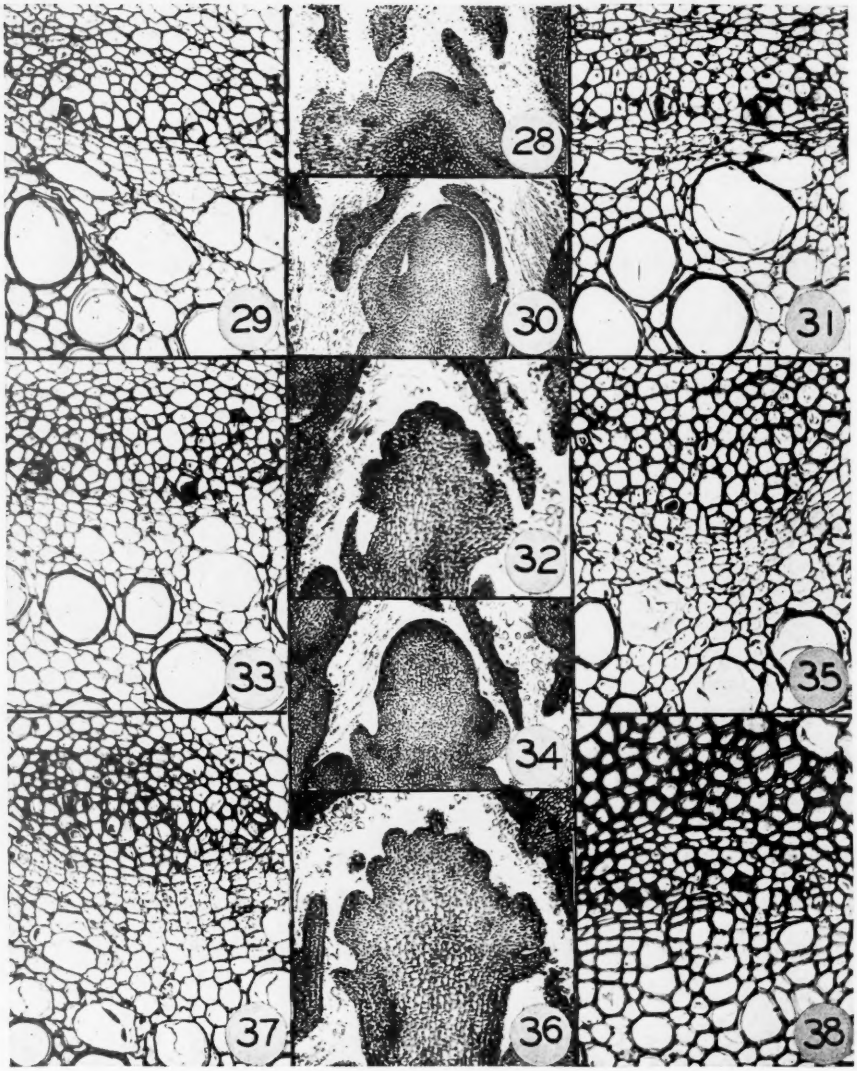
Cool temperature delayed the flowering of *Xanthium* on short days. Plants



FIGS. 8–17.—Stem tips and transections of fourth internode of soybean: Fig. 8, nonflowering plant; active cambium producing vascular tissue. Figs. 9, 10, 6 short days; no blossom primordia apparent, less active cambium, fewer xylem vessels, and phloem cells in process of differentiation. Figs. 11 (tip of axillary), 12, 12 short days; blossom primordia present, fewer meristematic and developing cells. Fig. 13, 5 short and 7 long days; 7 long days sufficient time for stem again to acquire characteristics of vegetative stem; cambium active. Figs. 14, 15, 39 short days; blossom buds present, meristematic tissue apparently absent in this internode. Fig. 16, 17 short and 22 long days; some cambial activity; plants given this treatment usually again acquire vegetative characteristics. Fig. 17, 24 short and 15 long days; cells of stem have become mature.



FIGS. 18–27.—Stem tips and transections of fourth internodes of stems. Figs. 18–22, *Cosmos:* Fig. 18, nonflowering plant; active cambium forming vascular tissue. Fig. 19, 3 short days; cambium somewhat less active than in vegetative stem. Figs. 20, 21, 12 short days; blossom primordia apparent, stem shows reduced cambial activity. Fig. 22, flowering plant; stem has no apparent meristematic tissue. Figs. 23–27, stock: Fig. 23, nonflowering plant; vegetative stem possesses features already described for other vegetative stems. Figs. 24, 25, 6 long cool days; no primordia apparent, slightly less active cambium. Figs. 26, 27, 30 long cool days; blossom buds conspicuous, stem possesses relatively inactive cambium.



FIGS. 28–38.—Stem tips and transections of fourth internode of *Xanthium:* Figs. 28, 29, nonflowering plant; cambium composed of several layers of meristematic cells; xylem vessels and phloem cells differentiating. Figs. 30, 31, 3 warm short days; no blossom primordia apparent, less active cambium. Figs. 32, 33, 7 short days; blossom primordia evident, reduction in amount of meristematic tissue. Figs. 34, 35, 9 cool short days; plant comparable in development to one given 3 warm short days. Figs. 36, 37, 20 cool short days; cool temperature delayed budding; structure of stem and tip comparable in development with the one given 7 warm short days. Fig. 38, fruiting plant; no apparent meristematic tissue, cells have become thick walled.

given 9 short days at a cool temperature were comparable in development with those given 3 short days at a warm temperature, and plants given 20 short days at a cool temperature possessed blossom primordia comparable with those given 7 short days at a warm temperature (figs. 34–38).

Stock is classified as a long-day plant. When grown at a cool temperature, however, it also blossoms on short days. It requires approximately 18 long days for blossom primordia to appear when grown at a medium temperature. In the long-day plants, differences in anatomical structure were apparent prior to the appearance of blossom primordia. Twenty-four and 30 days of long-day treatment resulted in the differentiation of blossom buds and further reduction in meristematic tissue (figs. 23–27).

From these observations it is evident that alterations in the vascular structure occur relatively early in the development of plants exposed to an environment favorable to flowering. What the significance of this might be is not yet known, but it is evident from the species examined that although different environmental conditions must be used to induce flowering, yet a similar internal structural development of the stem occurs.

The second question considered is: What is the effect of an environment favorable for vegetative growth upon plants previously exposed to an environment inducive to flowering? The vascular structure of the stems of those plants that had formed blossom primordia but which aborted when the plants were transferred to an environment favorable for vegetative growth was examined. Provided the exposure to the environment inducive to flowering had not brought about sufficient changes in anatomical structure, the cambium resumed its activity and differentiated new xylem and phloem elements. Soybean illustrates this condition. Plants given 12 short days possessed blossom primordia. Cross sections of stems indicated that the cambium was less active than in the vegetative stem, and the walls of the cells were becoming thickened. Phloem parenchyma instead of sieve tubes and companion cells were being formed. When comparing stems of plants given 12 short days with those that had received 12 days of treatment, 5 of which were short and 7 of which were long, it was found that the primordia were not yet apparent in the latter. The cambium in the stems of these plants was active, and vascular tissue was being differentiated (fig. 13). Twelve short days and 6 long days resulted in subsequent abortion of the blossom primordia and return of the plant to the vegetative condition. Another group of plants was given 39 days of environmental treatment. Some of these plants were exposed to a number of short days and then transferred to long days. Plants given 17 or more short days previous to their transfer to long days possessed blossom buds that matured into flowers and fruits. These plants had stems with little or no apparent meristematic tissue (figs. 16, 17).

Twelve long days followed by 12 short days were not favorable for producing blossom primordia in plants of stock.

When flowering plants of soybean, *Salvia*, *Cosmos*, and stock are desired, therefore, it is necessary to expose them to the environment inducive to flowering for a greater length of time than just the induction period required for the apparent changes in anatomical structure and the formation of blossom primordia. It is not sufficient to secure only primordia, for unless the environment is favorable for development of blossoms, abortion will occur.

The effects which the observed changes in anatomical structure prior to blossoming may have upon physiological relations have not yet been studied. The fact that comparable structural changes occur in plants at the time of blossom induction, without regard to the environmental conditions used to initiate the blossom state, would seem an added means of studying the nature of blossoming in flowering plants.

Summary

1. Soybean, *Salvia*, *Cosmos*, and *Xanthium*, all short-day plants, initiate blossom primordia after different durations of exposure to short days. Soybean and *Salvia* require 9, *Cosmos* 12, and *Xanthium* 5 days of short-day treatment for the appearance of blossom primordia at the appropriate temperature and cultural conditions. Stock, a long-day plant (at a medium or cool temperature), requires approximately 18 days for the appearance of primordia.

2. In order that the blossom primordia may continue to develop, it is necessary to expose the plants studied (except *Xanthium*) to a favorable environment for a greater length of time than just the induction period. *Xanthium* requires an induction period of only 2–3 short days at a warm temperature for the initiation and development of blossom buds. One short day is sufficient for the initiation of staminate blossoms.

3. Alterations in the anatomical structure of the stem occurred early in the development of plants placed in an environment favorable to the formation of blossom primordia. The first indication of a change in structure was a decrease in the number of meristematic cells of the cambial zone. Associated with this was the simultaneous decrease in the formation of xylem and phloem cells and a thickening of the cell walls of the vascular tissue. The cambium generally remained somewhat active, giving rise to new xylem but little phloem. The most recently formed phloem was composed almost entirely of phloem parenchyma cells. The stems of flowering and fruiting plants had little or no meristematic tissue, and the walls of the cells had become greatly thickened.

4. Plants of soybean, *Salvia*, *Cosmos*, and stock exposed to an environment favorable to flowering for a limited time only, and then returned to conditions

inducive to vegetative growth, soon showed renewed cambial activity and differentiation of vascular elements with the failure of the blossom primordia to continue development. Plants of *Xanthium* given 2–3 short days became reproductive regardless of subsequent treatment.

The writer expresses her appreciation to Professors R. H. ROBERTS and EMMA L. FISK for valued suggestions and constructive criticism generously contributed throughout the progress of this investigation.

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SIROCLADIUM, A NEW TERRESTRIAL MEMBER OF THE ZYGNEMALES

M. S. RANDHAWA

(WITH FIFTEEN FIGURES)

This alga was collected by the writer from the Kumaon Hills, at an altitude of about 5500 feet, in September, 1939. It was found on soft clay under an overhanging rock, at a distance of about 10 feet from a small waterfall, growing in the form of dark green feltlike radial patches about 1–2 cm. in diameter. Unlike some terrestrial species of *Vaucheria* and *Oedogonium terrestris*, it was never found in the form of mats. The moist clay on which it was growing received a light spray from the waterfall. The alga was shaded by an overhanging rock on the north and by the shade of a tree on the south, and was also protected by another rock, so that it never received direct sunlight.

Observations

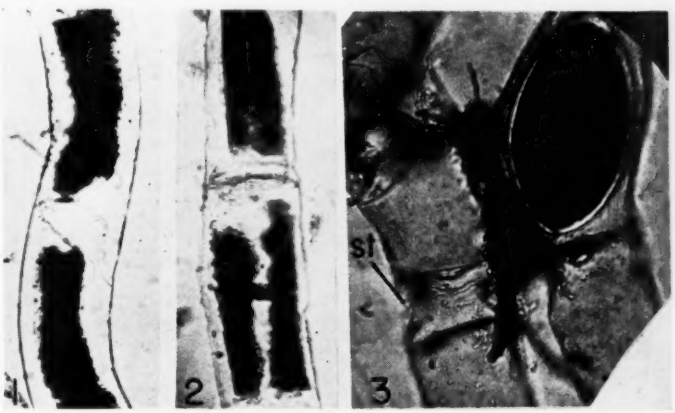
CHLOROPLASTS.—The cells are rectangular in outline, 45–64μ broad and 120–210 μ long. Septa are plane. Most of the subaerial cells have two flat, axile, plate-shaped chloroplasts, each of which is as broad as the cells, after allowing for thickness of cell walls. These two chloroplasts lie parallel to each other laterally, and in most cases cover each other so completely that the cells seem to contain only a single chloroplast (figs. 1, 2, 6), and are indistinguishable from those of broad species of *Mougeotia*.

The margin of these platelike chloroplasts is usually irregular, and each bears a number—4–17 as a rule—of conspicuous pyrenoids, scattered irregularly (figs. 4–6). In some cases the pyrenoids are surrounded with rings of starch particles (fig. 4). The broad platelike chloroplasts completely fill the interior of the cells in fresh material, and considerable shrinkage takes place, even when preserved in 4 per cent formalin.

In cells close to the soil the chloroplasts are reduced in size and are 20–30 μ broad; that is, approximately half the size of the cells. When seen edgewise these chloroplasts appear as linear, threadlike bodies. In some cases these narrow plates overlap in such a manner that they simulate a single plate and their double nature is seen only by a change of focus under the high power. As one follows the cells into the soil, a gradual reduction is noticed in the size of the two platelike chloroplasts, which are ultimately reduced to two linear threadlike chloroplasts joined together by a cytoplasmic isthmus in which a pale blue conspicuous nucleus is

seen. Pyrenoids in these reduced chloroplasts are also considerably attenuated (figs. 10–15). Further down only fragments of chloroplasts remain in some cells, while the others are almost empty.

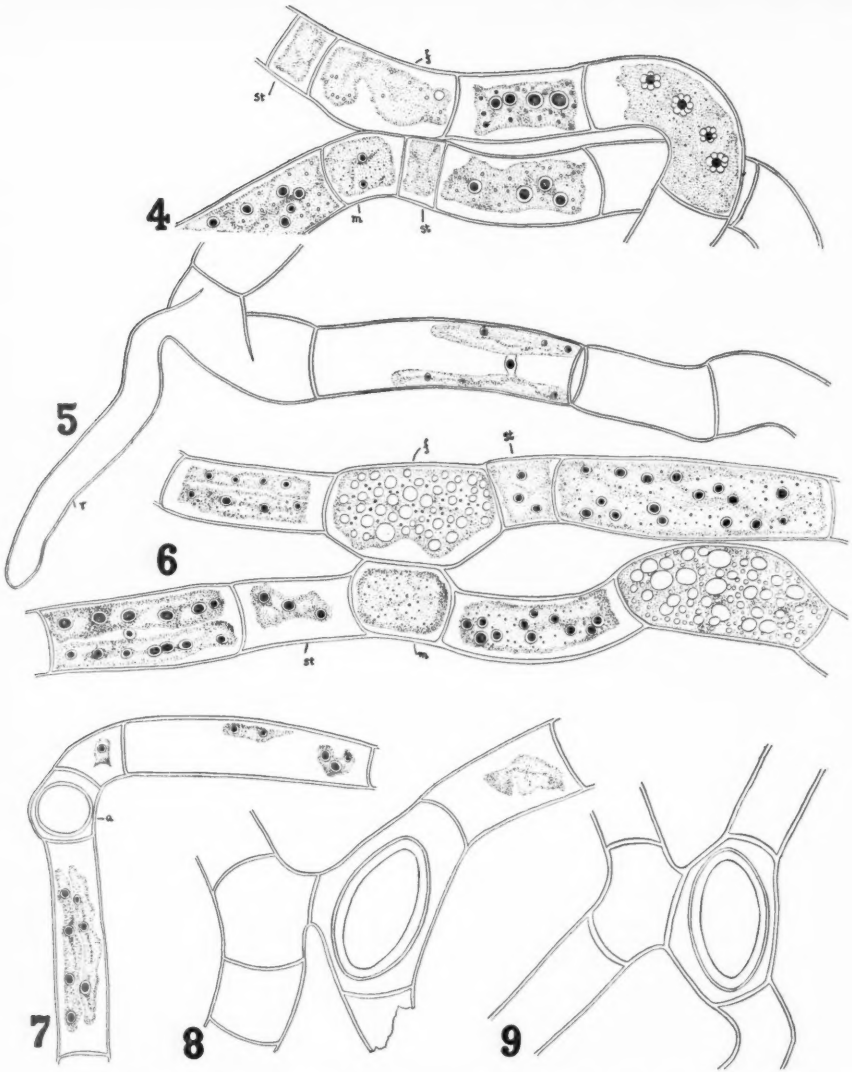
RHIZOIDS.—The subterranean cells are usually much elongated and are narrower as compared with the subaerial cells, as in most terrestrial algae. In some cases the terminal cells are drawn out into rhizoidal structures (figs. 12, 15), and occasionally even an intercalary cell gives out a rhizoidal structure laterally (fig. 5). Some of the intercalary cells which happen to get buried under soil particles



FIGS. 1–3.—*Sirocladium kumaoensis* gen. et sp. nov. Figs. 1, 2, subaerial cells showing chloroplasts. Considerable shrinkage of protoplasm and chloroplasts occurred in this preserved material. Fig. 3, ripe zygospore and sterile cell (*st*).

lengthen considerably and serve a rhizoidal function (figs. 13, 14). Even branching may be observed in some of the rhizoidal cells (fig. 10).

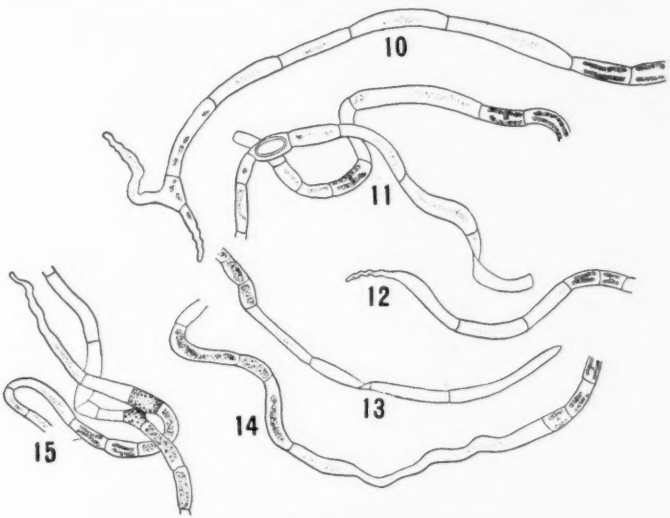
CONJUGATION.—The mode of conjugation in *Sirocladium* is similar to that of *Sirogonium*. Filaments in all stages of conjugation were collected on September 7, and mature zygospores were seen in abundance on September 23 and also October 17, the last collection. There is a noticeable difference in the size of male and female cells, and the latter are loaded with starch and other food materials. The filaments are usually much more coiled in conjugating than in sterile material, and probably this helps in the process by providing points of contact which stimulate tactile responses. As in *Sirogonium*, only a single sterile cell is cut off from the female cell, while one or two sterile cells are cut off from the cell which ultimately functions as the male (figs. 4, 6). Mucilaginous pads are seen at the point of contact between the mating cells (fig. 3). As in *Sirogonium*, conjugation is of a typically geniculate type, although in some cases lateral cell walls may be lengthened



FIGS. 4–9.—Fig. 4, early stage in conjugation; sterile cells (*st*) cut off from male (*m*) and female (*f*) cells. Fig. 5, lower part of filament showing attenuated chloroplasts and rhizoid (*r*). Fig. 6, later stage in conjugation showing increased size of male and female cells and different types of chloroplasts. Fig. 7, an azygospore (*a*). Figs. 8, 9, conjugation and ripe zygospores.

at the point of junction, simulating a tube (fig. 8). Zygospores are ellipsoid in shape, 42–70μ broad and 100–118μ long. Ripe zygospores are yellowish brown in color, with a smooth mesospore. Conjugation takes place not only among subaerial cells, but in some cases it was noticed that even underground cells conjugated and produced ripe zygospores. In such cases the conjugating cells as well as the neighboring ones do not contain chloroplasts, although they are somewhat rich in protoplasm (fig. 8).

AZYGOSPORES.—As in certain species of *Spirogyra* and *Zygnema*, the contents of the male cells in some instances develop into rounded azygospores (fig. 7). This



FIGS. 10–15.—Different types of rhizoids

may be due to the fact that after the formation of a male cell has been induced by contact with another filament, that filament is displaced and moves apart, owing to action of air or impact with drops of water.

Discussion

So far as the mode of conjugation is concerned, *Sirocladium* resembles *Sirogonium;* there is the same elimination of conjugation canals and inequality in the size of the conjugating cells. In the structure of its chloroplasts, however, this alga resembles certain broad species of *Mougeotia*, and in its habit, species of *Zygogonium* and *Zygnema terrestris* (2). More minutely considered, the chloroplasts of *Sirocladium* are unique; while chloroplasts of *Mougeotia* are axile, in this alga the two broad plates are lateral in position.

Only a system of classification of Zygnemales which takes into consideration both the structure of chloroplasts as well as the modes of reproduction can be regarded as satisfactory (3). CZURDA (1) bases the genera of Zygnemales on the structure of chloroplasts alone, but this concept involves difficulties and inconsistencies. According to such a system it is not difficult to group together forms like *Zygnema*, *Zygogonium*, *Pleurodiscus*, and *Zygnemopsis* into an all-embracing genus *Zygnema;* and *Mougeotia*, *Debarya*, and *Temongametum* into *Mougeotia;* but an alga like the present one presents a problem. If CZURDA's system of classification is followed, the present form must be placed close to *Mougeotia*. If only the mode of reproduction is considered, widely diverse forms will be brought together; for example, species of both *Zygnema* and *Spirogyra* show anisogamous conjugation, which hardly justifies grouping them together in the same genus. Following the same principle, the mere presence of geniculate conjugation and absence of conjugation canals do not justify the inclusion of the present alga in genus *Sirogonium*. When the alga was originally collected, in a sterile condition in early September, the broad platelike chloroplasts of the subaerial cells indicated a terrestrial species of *Mougeotia*, and only when conjugating cells were found was its affinity with *Sirogonium* suggested. Moreover, no terrestrial species of *Sirogonium* is known so far, and platelike chloroplasts are not known in any species of *Sirogonium*. It is true that the reduced chloroplasts in some of the subterranean cells show resemblances with some species of *Sirogonium*, in which the number of chloroplasts is few, but even then the resemblance is superficial, for there is no known species of *Sirogonium* in which the number of chloroplasts is reduced to the fixed number of two. In nearly all the species of *Sirogonium* the number of chloroplasts is usually 5–8 or more, and only in a few cells are they reduced to one or two on account of certain physiological factors. Its peculiar terrestrial habitat, its varied chloroplasts, and its mode of conjugation, justify the formation of a new genus of the Zygnemales, which is named *Sirocladium*.

Sirocladium takes its place in the family Mougeotiaceae of the order Zygnemales, next to *Sirogonium*. So far as its terrestrial habit and presence of rhizoids is concerned, it is certainly a more advanced and specialized form than *Sirogonium*. It is futile to make any generalization about the comparative primitiveness or advanced nature of axile platelike chloroplasts as compared with semi-spiral or straight bandlike chloroplasts, for each represents an adaptation toward particular environmental conditions.

Sirocladium gen. nov.

Filaments usually simple with lowest cells drawn out into rhizoids, rarely with lateral branches of a few cells in the subterranean part. Cells cylindrical with plane septa, each containing two lateral plate-shaped chloroplasts which in cells

close to the soil or partly subterranean are reduced to two bandlike wavy bodies. Conjugation direct between genuflexed cells, without the intermediation of conjugation tubes. One or more sterile cells cut off from both female and male cells, the former considerably larger than the latter. Zygospores ellipsoid. The only recorded species is terrestrial in habit.

Sirocladium kumaoensis sp. nov.

Characters as those of the genus.

Vegetative cells 45–64μ broad and 120–210μ long; fertile cells inflated up to 90μ in diameter; zygospores ellipsoid, 42–68μ diameter, 102–118μ long; spore wall smooth, yellowish brown.

Growing on moist clay under a shady rock in Kumaon Hills, Himalayas, Almora U.P., India, in September and October, 1939.

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CHROMOSOMES OF SOME ALISMACEAE

ELEANOR MARIE OLESON

(WITH NINE FIGURES)

Introduction

LIEHR (1) determined the diploid chromosome number of *Alisma plantago* L. as 12. WULFF (10) reported it as 10, but was uncertain as to the accuracy of his count since the chromosomes are long and twisted.

LIEHR (1) reported 16 as the diploid number in *Sagittaria sagittifolia* L. LOHAMMAR (2), however, found 22 in this species, as well as in *S. natans* Pallas. According to LOHAMMAR, there is little cytological difference between the two species; while the chromosomes differ somewhat in size, the idiograms of the two are similar. In both species there are two long chromosomes with central fiber-attachment constrictions. One, possibly two, have primary constrictions at approximately one-third the distance from one end. The other chromosomes have subterminal constrictions. In the metaphases they are long and twisted. TAYLOR (8) reported 20 as the apparent diploid number in *S. montevidensis* Cham. and Schlecht. but found difficulty in counting the long twisted chromosomes. In two, possibly four, of the chromosomes the primary constrictions are median; in the others they are subterminal.

NAWA (5) found 20 to be the diploid number in *S. trifolia* var. *sinensis* (Sims) Makino. For the same species, MORINAGA and FUKUSHIMA (3) reported 11 as the haploid number and 22 as the diploid. According to the latter investigators there are 2 long chromosomes with median fiber attachments; 2 have subterminal and 18 have terminal fiber attachments. In *S. aginashi* Makino, SHINKE (6, 7) determined the haploid number as 11, the diploid as 22. The chromosomes fall roughly into four groups with respect to size and position of fiber-attachment points. Two long chromosomes have central fiber-attachment constrictions. Two very short chromosomes are rod-shaped, the position of the fiber-attachment points not being designated. The remaining 18 are rod-shaped with subterminal primary constrictions. They are divided into two groups; in one the constrictions are clear and deep, in the other group they are obscure. The internal spiral structure of the meiotic chromosomes was studied in detail. NARASIMHA MURTHY (4) found the haploid number in *Limnophyton obtusifolium* (L.) Miq. to be 12.

Table 1 gives the chromosome numbers reported for members of this family, including the results of the present study.

Material and methods

During the summer months of 1939, root tips and buds of *Alisma plantago* L., *Sagittaria latifolia* Willd., and *S. rigida* Pursh. were taken from plants growing in marshes in Columbia County, Wisconsin, and immediately fixed, using Flemming's medium solution and Belling's and Randolph's modifications of Navashin's formula. Sections were cut at 10 μ.

Material fixed in the Flemming solution was stained with Heidenhain's iron-alum haematoxylin. The crystal violet-iodine stain followed by picric acid was used with a portion of the material fixed in Belling's and Randolph's solutions.

TABLE 1

Species	n	$2n$	Investigator
Alisma plantago	.....	12	Liehr, 1916
A. plantago	.....	10	Wulff, 1939
A. plantago	.....	14	Oleson
Limnophyton obtusifolium	12	.....	Narasimha Murthy, 1933
Sagittaria aginashi	11	22	Shinke, 1929, 1934
S. latifolia	11	22	Oleson
S. montevidensis	.....	20	Taylor, 1925
S. natans	.....	22	Lohammar, 1931
S. rigida	.....	22	Oleson
S. sagittifolia	.....	16	Liehr, 1916
S. sagittifolia	.....	22	Lohammar, 1931
S. trifolia var. sinensis	.....	20	Nawa, 1928
S. trifolia var. sinensis	11	22	Morinaga and Fukushima, 1931

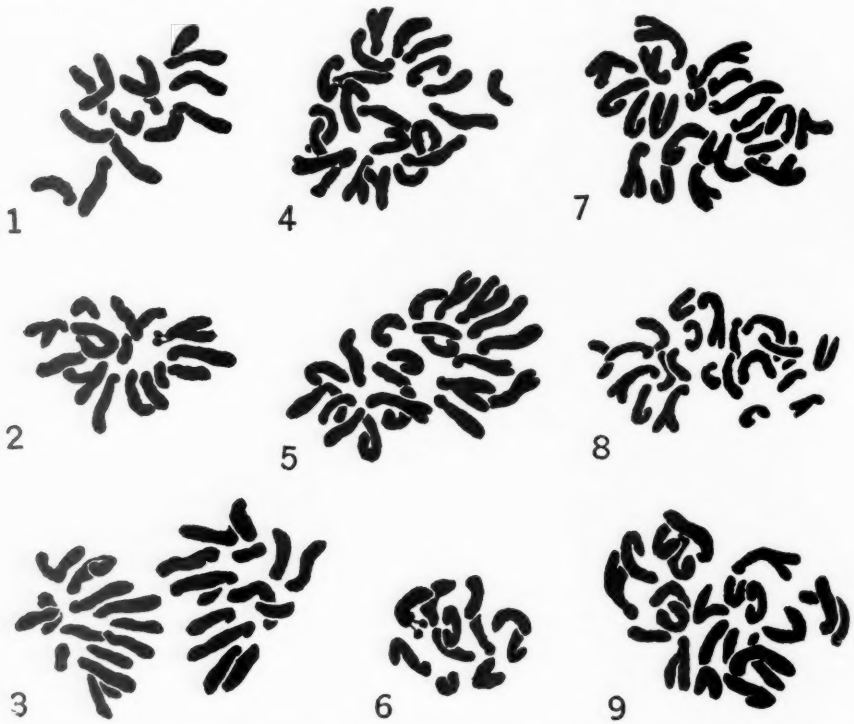
La Cour's crystal violet-iodine schedule was used for the remainder of the material. Material fixed in Belling's solution and stained by La Cour's method was found most satisfactory for the study of chromosomes.

Observations

The somatic chromosome number in *Alisma plantago* (figs. 1–3) is 14. This number does not agree with that reported either by Liehr ($2n = 12$) or by Wulff ($2n = 10$). For this reason the count here given was decided upon only after a considerable number of favorable equatorial plates and anaphase figures were examined and drawn.

The chromosomes are long and twisted in equatorial-plate figures. They may be classed as long, medium, and short. Primary constrictions are of two types, median and subterminal. Four chromosomes are long, 2 having median and 2 subterminal constrictions. The remaining 10 have subterminal constrictions; 6 of these are of medium length and 4 are short. The primary constrictions are conspicuous in most cases. Secondary constrictions are generally obscure and were observed in only a few instances. In one equatorial plate (fig. 2) an already

divided satellite is attached to the short arm of one of the short chromosomes. The length of the attaching fiber equals approximately the diameter of the chromosome body. The homologous chromosome can be identified, but it is in a crowded position and the presence of a satellite cannot be determined.



FIGS. 1–9.*—Fig. 1, *Alisma plantago*, equatorial plate. Fig. 2, same showing one short chromosome with divided satellite. Fig. 3, same showing anaphase with 14 chromosomes in each group. Figs. 4, 5, *Sagittaria latifolia*, equatorial plate. Fig. 6, same, microspore. Metaphase showing one chromosome with divided satellite. Figs. 7, 8, 9, *S. rigida*, equatorial plate.

* Drawings made with camera lucida, using Spencer 1.5 mm. objective and compensation ocular 12×. All except fig. 6 are from cells of root tips. × 2200.

The haploid chromosome number in *Sagittaria latifolia* (fig. 6), determined from division figures in microspores, is 11. Five chromosomes are long, 4 of medium length, and 2 short. Primary constrictions are commonly obscure. Two long chromosomes have median constrictions; in the others the constrictions are subterminal. One short chromosome has been seen to have a rather large divided satellite attached to the short arm (fig. 6).

Twenty-two chromosomes appear during mitosis in root-tip cells (figs. 4, 5). They are long and twisted in equatorial plates. Ten chromosomes are long, 8 of medium length, and 4 short. Primary constrictions are usually conspicuous and deep. Four of the long chromosomes have median constrictions; the other 18 have subterminal constrictions. Secondary constrictions are visible in only a few cases. Satellites were not observed in somatic cells.

In *Sagittaria rigida* (figs. 7–9) 22 chromosomes are present in somatic cells. The chromosomes are of approximately the same size and the same general appearance as in *S. latifolia*. The primary constrictions are commonly distinct. Ten chromosomes are long, 2 having median and 8 subterminal constrictions. Eight chromosomes are of medium length, 2 having median and 6 subterminal constrictions. There are 4 short chromosomes with subterminal constrictions. Secondary constrictions appear in the long arms of a few chromosomes with subterminal primary constrictions. Satellites were not observed.

It is suggested that the basic chromosome numbers of the Alismaceae thus far studied are 7 in *Alisma*, 12 in *Limnophyton*, and 11 in *Sagittaria*. Although diploid numbers of 16 and 20 have been reported for certain species of *Sagittaria*, and 12 and 10 by previous observers for *A. plantago*, there is some uncertainty as to the accuracy of these numbers. My observations on the last-named species seem to indicate that previous counts were erroneous, unless varieties occur with different numbers.

Summary

1. The diploid chromosome number in *Alisma plantago* is 14. In *Sagittaria latifolia* the haploid chromosome number is 11, the diploid number 22. The diploid chromosome number in *S. rigida* is 22.

2. The chromosomes of these three species are similar in size and appearance. Primary constrictions are of two types, median and subterminal. The chromosomes may be distinguished as long, medium, and short.

3. Satellite chromosomes of *A. plantago* and *S. latifolia* show the same general characteristics as to length of chromosome body, position of the primary constriction, and size of satellites.

4. Basic chromosome numbers are probably 7 in *Alisma*, 12 in *Limnophyton*, and 11 in *Sagittaria*.

The writer is indebted to Dr. C. E. Allen, under whose direction this work was done.

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CURRENT LITERATURE

Experimentelle Cytologie. By HANS H. PFEIFFER. A New Series of Plant Science Books, edited by Frans Verdoorn. Vol. IV. Waltham, Mass. (formerly Leyden): Chronica Botanica Co., 1940. Pp. 243. Figs. 28. $4.00.

This text constitutes a survey of the scope, methods, and results of contemporary research in experimental plant cytology. The purpose of Dr. PFEIFFER is to fill a gap in plant science literature. He admits, however, that an exhaustive review of experimental work on plant cells with its diverse viewpoints cannot be presented in such a small volume; many results of a purely chemical, physical, and genetical nature are therefore omitted. Even so, a vast amount of literature concerning the application of experiment to problems of cellular and tissue development has been assembled, and many phenomena have been correlated and presented. Selection, arrangement, and theoretical treatment of the material reflect the author's previous methodologic-analytical efforts and are predominantly based on his own research and on that of KÜSTER, FREY-WYSSLINGH, and other European investigators of normal and pathological plant and cell structure.

The first three chapters are introductory, dealing with the scope of experimental cytology: the material, methods, and agents used in the experimental production of cellular reactions or formation of cell structures, and form and size changes of the cell, nucleus, and plastids. Chapters follow on the basic physiological properties of protoplasm and the living cell, such as structure, physico-chemical and biological properties of protoplasm; osmosis; permeability; viscosity; vital staining; electrophysiological experiments; ionic, pH, and radiation effects.

In the remaining portion changes in visible cell structure and typical cellular functions, as well as the submicroscopical processes underlying them, are discussed in relation to different experimental treatments, in chapters on cell growth; differentiation, dedifferentiation and necrosis; explantation of cells; mitosis; cytokinesis; dislocation experiments; micrurgy; extraction and introduction of cellular components and substances.

Emphasis is laid on the general morphological and physiological behavior of the cell rather than on cell division, the nucleus and the chromosomes. The attitude is analytical and comparative throughout, with no preference for any particular experimental method, and—though the treatment is often somewhat sketchy—the danger of oversimplifying complex phenomena has been avoided. Lists of important papers and texts are given, but unfortunately citations for most of the papers mentioned in the text are omitted. Several topics, which the student of present-day cytology might expect to find, are not discussed, such as effects on the cell of natural and synthetic growth substances, enzymes, and colchicine, as well as the work on nucleic-acid metabolism, polyploidy, and tumor production. In addition to the author and subject index, there is a survey of important cytological contributions from 1748 to 1939.

The volume should prove useful in connection with the present standard texts of general and comparative plant cytology, none of which has yet synthesized our somewhat unconnected knowledge in the various fields of experimental cytology. Investigators will find it helpful as an outline of experimental methods and general results, and for its many references; it should prove stimulating to the students of morphology and morphogenesis, and to the general cytologist it may serve as a botanical supplement to JAMES GRAY'S textbook.—ROBERT BLOCH.